PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 30 June 1997 (30.06.97)	
International application No. PCT/BE96/00123	**Applicant's or agent's file reference** P.SCRE.01/WO
International filing date (day/month/year) 21 November 1996 (21.11.96)	**Priority date** (day/month/year) 21 November 1995 (21.11.95)
Applicant COMMUNI, Didier et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

16 June 1997 (16.06.97)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer B. Fitzgerald Telephone No.: (41-22) 338.83.38

ATENT COOPERATION TREA

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:
VAN MALDEREN Michel
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
1083 Bruxelles
BELGIQUE

REÇU
26-6-1997
OFFICE VAN MALDEREN

NOTIFICATION OF RECEIPT OF DEMAND

(PCT Rule 61.1(b), first sentence and Administrative Instructions, Section 601)

Date of mailing *(day/month/year)*	24.06.97
Applicant's or agent's file reference: P.SCRE.01/WO	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/BE 96/00123	21/11/1996	21/11/1995

Applicant

EUROSCREEN S.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

16/06/1997

2. This date of receipt is:

☒ the actual date of receipt of the demand.

☐ the date on which the proper corrections to the demand were timely received.

3. ☐ This date is **AFTER** the expiration of 19 months from the priority date.

Attention: The election(s) made in the demand does (do) not have the effect of postponing the commencement of the national phase until 30 months from the priority date (or later in some Offices)(Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22).

For details, see Annex B to Form PCT/IB/301 sent by the International Bureau and Volume II of the PCT Applicant's Guide.

☐ This notification confirms the information given in person or by telephone on:

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	N. Werner Telephone No. Nadine Werner

WO 97/19170
PCT/BE96/00123

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

VAN MALDEREN, Michel
Office Van Malderen
Place Reine-Fabiola 6/1
B-1083 Bruxelles
BELGIQUE

REÇU
- 6. -6- 1997
OFFICE VAN MALDEREN
EVM

Date of mailing (day/month/year) 29 May 1997 (29.05.97)	
Applicant's or agent's file reference P.SCRE.01/WO	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/BE96/00123	21 November 1996 (21.11.96)	21 November 1995 (21.11.95)

Applicant
EUROSCREEN S.A. et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   CA,EP,JP,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   None

   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   29 May 1997 (29.05.97) under No. WO 97/19170

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 730.91.11

Continuation of Form PCT/IB/30[illegible]

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

Date of mailing (day/month/year) 29 May 1997 (29.05.97)	**IMPORTANT NOTICE**
Applicant's or agent's file reference P.SCRE.01/WO	**International application No.** PCT/BE96/00123

The applicant is hereby notified that, at the time of establishment of this Notice, the time limit under Rule 46.1 for making amendments under Article 19 has not yet expired and the International Bureau had received neither such amendments nor a declaration that the applicant does not wish to make amendments.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

PCT/BE96/00123
International Application No.

21 NOV.1996 (21-11-1996)
International Filing Date

RO/BE - PCT INTERNATIONAL APPLICATION
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) P.SCRE.01/WO

Box No. I TITLE OF INVENTION

RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR.

Box No. II APPLICANT

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

EUROSCREEN S.A.
Avenue des Becassines 7
B-1160 BRUXELLES
BELGIUM

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

COMMUNI Didier
Groendallaan 19
B-1800 VILVOORDE
BELGIUM

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name: for a legal entity, full official designation. The address must include postal code and name of country.)*

Michel VAN MALDEREN
OFFICE VAN MALDEREN
Place Reine Fabiola 6/1
B-1083 BRUXELLES
BELGIUM

Telephone No. 32-2-4263810

Facsimile No. 32-2-4263760

Teleprinter No. 63628 patbel

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

PIROTTON Sabine
Avenue Marius Renard 27a
B-1070 BRUXELLES
BELGIUM

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

PARMENTIER Marc
Chaussée d'Uccle 304
B-1604 LINKEBEEK
BELGIUM

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

BOEYNAEMS Jean-Marie
Avenue Peter Benoît 5
B-1780 WEMMEL
BELGIUM

This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BE

State *(i.e. country)* of residence: BE

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

Use this box in the following cases:

1. If, in any of the Boxes, the space is insufficient to furnish all the information:

in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available:

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents:

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":

(vi) if there are more than three earlier applications whose priority is claimed:

in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;

in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;

in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.

2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:

in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.

Continuation of Box No. IV
OTHER REPRESENTATIVES
Joëlle VAN MALDEREN
Eric VAN MALDEREN

Place Reine Fabiola 6/1
B-1083 BRUXELLES
BELGIUM

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☐ **AP** **ARIPO Patent:** **KE** Kenya, **LS** Lesotho, **MW** Malawi, **SD** Sudan, **SZ** Swaziland, **UG** Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☐ **EA** **Eurasian Patent:** **AM** Armenia, **AZ** Azerbaijan, **BY** Belarus, **KG** Kyrgyzstan, **KZ** Kazakstan, **MD** Republic of Moldova, **RU** Russian Federation, **TJ** Tajikistan, **TM** Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** **European Patent:** **AT** Austria, **BE** Belgium, **CH and LI** Switzerland and Liechtenstein, **DE** Germany, **DK** Denmark, **ES** Spain, **FI** Finland, **FR** France, **GB** United Kingdom, **GR** Greece, **IE** Ireland, **IT** Italy, **LU** Luxembourg, **MC** Monaco, **NL** Netherlands, **PT** Portugal, **SE** Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☐ **OA** **OAPI Patent:** **BF** Burkina Faso, **BJ** Benin, **CF** Central African Republic, **CG** Congo, **CI** Côte d'Ivoire, **CM** Cameroon, **GA** Gabon, **GN** Guinea, **ML** Mali, **MR** Mauritania, **NE** Niger, **SN** Senegal, **TD** Chad, **TG** Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☐ **AL** Albania .....
- ☐ **AM** Armenia .....
- ☐ **AT** Austria .....
- ☐ **AU** Australia .....
- ☐ **AZ** Azerbaijan
- ☐ **BB** Barbados
- ☐ **BG** Bulgaria .....
- ☐ **BR** Brazil .....
- ☐ **BY** Belarus
- ☒ **CA** Canada
- ☐ **CH and LI** Switzerland and Liechtenstein
- ☐ **CN** China .....
- ☐ **CZ** Czech Republic .....
- ☐ **DE** Germany .....
- ☐ **DK** Denmark .....
- ☐ **EE** Estonia .....
- ☐ **ES** Spain .....
- ☐ **FI** Finland .....
- ☐ **GB** United Kingdom
- ☐ **GE** Georgia .....
- ☐ **HU** Hungary .....
- ☐ **IL** Israel
- ☐ **IS** Iceland
- ☒ **JP** Japan .....
- ☐ **KE** Kenya .....
- ☐ **KG** Kyrgyzstan .....
- ☐ **KP** Democratic People's Republic of Korea .....
- ☐ **KR** Republic of Korea .....
- ☐ **KZ** Kazakstan .....
- ☐ **LK** Sri Lanka
- ☐ **LR** Liberia
- ☐ **LS** Lesotho .....
- ☐ **LT** Lithuania
- ☐ **LU** Luxembourg .....
- ☐ **LV** Latvia
- ☐ **MD** Republic of Moldova .....
- ☐ **MG** Madagascar .....
- ☐ **MK** The former Yugoslav Republic of Macedonia .....
- ☐ **MN** Mongolia
- ☐ **MW** Malawi .....
- ☐ **MX** Mexico .....
- ☐ **NO** Norway
- ☐ **NZ** New Zealand .....
- ☐ **PL** Poland .....
- ☐ **PT** Portugal .....
- ☐ **RO** Romania
- ☐ **RU** Russian Federation .....
- ☐ **SD** Sudan
- ☐ **SE** Sweden
- ☐ **SG** Singapore
- ☐ **SI** Slovenia .....
- ☐ **SK** Slovakia .....
- ☐ **TJ** Tajikistan .....
- ☐ **TM** Turkmenistan .....
- ☐ **TR** Turkey .....
- ☐ **TT** Trinidad and Tobago .....
- ☐ **UA** Ukraine .....
- ☐ **UG** Uganda .....
- ☒ **US** United States of America .....
- ☐ **UZ** Uzbekistan .....
- ☐ **VN** Viet Nam .....

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- ☐ .....
- ☐ .....
- ☐ .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____.

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) EP	(21/11/1995) 21 November 1995	95870124.5	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

[X] The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s): ~~(1)~~ 1

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / OEB

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request.*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	5	sheets
2. description	27	sheets
3. claims	13	sheets
4. abstract	1	sheets
5. drawings	12	sheets
Total	58	sheets

This international application is accompanied by the item(s) marked below:

1. [X] separate signed power of attorney
2. [ ] copy of general power of attorney
3. [ ] statement explaining lack of signature
4. [ ] priority document(s) *identified in Box No. VI as item(s):*
5. [X] fee calculation sheet
6. [ ] separate indications concerning deposited microorganisms
7. [ ] nucleotide and/or amino acid sequence listing (diskette)
8. [ ] other *(specify)*:

Figure No. _____ of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Michel VAN MALDEREN

For receiving Office use only

1. Date of actual receipt of the purported international application: 21 NOV 1996 (21-11-1996)
2. Drawings: [X] received: [ ] not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
6. [ ] Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

INTERNATIONAL SEARCH REPORT

Inter[illegible]al Application No
PCT/BE 96/00123

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 . C12N15/12 C07K14/705 C12N15/85 C12N15/86 C12N5/10
C12Q1/68 C07K16/28 A01K67/027 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q A01K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, April 1994, pages 3275-3279, XP000611412 PARR C E ET AL: "CLONING AND EXPRESSION OF A HUMAN P2U NUCLEOTIDE RECEPTOR, A TARGET FOR CYSTIC FIBROSIS PHARMACOTHERAPY" cited in the application see the whole document --- -/--	11-14, 16-19, 21-23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

12

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 1997	02. 05. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 95 10538 A (UNIV NORTH CAROLINA ;UNIV MISSOURI (US); BOUCHER RICHARD C (US); W) 20 April 1995 see the whole document	14-19, 21-25 1-13, 26-28, 35,36, 38-40, 47-67, 76,77
A	TRENDS IN PHARMACOLOGICAL SCIENCES, vol. 16, April 1995, pages 133-139, XP002030122 BOARDER M. ET AL.: "G protein-coupled P2 purinoceptors: from molecular biology to functional responses" * see the whole document, esp. p. 137 *	1-77
A	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 16, 22 April 1994, pages 11830-11836, XP002030123 LAZAROWSKI E. AND HARDEN T.: "Identification of a uridine nucleotide-selective G-protein-linked receptor that activates phospholipase C" cited in the application see the whole document	1-77
P,X	FEBS LETTERS, vol. 384, no. 3, 22 April 1996, pages 260-264, XP002030124 STAM N. ET AL.: "Molecular cloning and characterization of a novel orphan receptor (P2p) expressed in human pancreas that shows high structural homology to the P2u purinoceptor" see the whole document	1-16,24, 25,27
P,X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 52, 29 December 1995, pages 30849-30852, XP002030125 COMMUNI D. ET AL.: "Cloning and functional expression of a human uridine nucleotide receptor" see the whole document	1-23
T	WO 96 38558 A (INCYTE PHARMA INC) 5 December 1996 see the whole document	1-77

12

INTERNATIONAL SEARCH REPORT

onal application No.
PCT/BE 96/00123

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 70,73
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although these claims are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...mation on patent family members

...al Application No
PCT/BE 96/00123

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9510538 A	20-04-95	AU 7965694 A	04-05-95
		US 5596088 A	21-01-97
		US 5607836 A	04-03-97
WO 9638558 A	05-12-96	AU 5972996 A	18-12-96
		AU 6032596 A	18-12-96
		WO 9638591 A	05-12-96

PATENT COOPERATION TREATY

28 Rec'd PCT/PTO 21 MAY 1998

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
P.SCRE.01/WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/BE96/00123	21/11/1996	21/11/1995
International Patent Classification (IPC) or national classification and IPC		
C12N15/12		
Applicant		
EUROSCREEN S.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 11 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/06/1997	03.03.98
Name and mailing address of the IPEA/	Authorized officer
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Form PCT/IPEA/409 (Cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE96/00123

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-27	as originally filed				

Claims, No.:

1-69	as received on	06/01/1998	with letter of	06/01/1998	

Drawings, sheets:

1/12-12/12	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inv ntive st p or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-23,26-28,30-48,50,52,57-62,64,65,67-69
	No:	Claims	24, 25, 29, 49, 51, 53, 54, 63, 66
Inventive step (IS)	Yes:	Claims	1-23,26-28,31-48,58-62,68,69
	No:	Claims	24, 25, 29, 30, 49-57, 63-67
Industrial applicability (IA)	Yes:	Claims	1-69
	No:	Claims	

2. Citations and explanations

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

SECTION V ----------

1). This report is established on the assunmption that the present application enjoys a valid priority. In case of an invalid priority, documents "FEBS LETTERS, vol. 384, no. 3, 22.04.1996, p. 260-264, Stam,N. et al." and "The J. of Biol. Chem., vol. 270, no. 52, 29.12.1995, p. 30849-30852, Communi,D. et al." may become relevant in the european phase.
This is also true for document WO 96/38558 which has an earlier priority (02.06.1995) and which may become relevant for those claims which refer to a ligand for the receptor other than nucleotides, in particular an antibody.

2). The new set of claims 1-69 appears to fulfil the requirement of Art. 34, 2(b) PCT that the amendments shall not go beyond the disclosure in the international application as filed.

3). It would further appear that claims 24, 25, 49, 51, 53, 54, 56, 63 and 66 are not novel and inventive and claims 52, 57, 64 and 67 are not inventive within the meaning of Art. 33(2), (3) PCT.
Document "Proc. NAt. Acad. Sci., vol. 91, 1991, 3275-3279, Parr,C.E. et al." describes in particular on page 3278 and Fig. 4 the use of probe D9 consisting of an about 500 bp portion of the P2U receptor coding sequence in a Northern blot analysis. This probe is considered to anticipate the novelty of the probe of claims 24 and 25 because the "unique sequence" on the receptor encoding nucleic acid molecule is not defined. D9 appears to comprise a stretch of at least 15 nucleotides which can specifically hybridize with the nucleic acid molecule encoding the present receptor.
The subject-matter of claims 29, 56, 63 and 66 is not defined by a single

technical feature. Therefore, the compounds described in the above identified document such as carbachol (page 3278, Fig. 3B) and pertussis toxin (page 3276) are considered to fall within the scope of said claims. It is further noted that the present application makes reference to "antagonists" whereby PPADS, a substance obviously already known in the art appeared to be the most active antagonist of the present receptor.

The dependent claims 30, 57, 64 and 67 do not contain additional features suitable to render the product claimed inventive in the sense of Art. 33(3) PCT.

In view of the above mentioned parts in the cited document, the same objections obviously also apply to the method claims 49, 51, 53 and 54 which do not appear to be novel and to claims 50 and 52 which are not considered to be inventive.

4). The remaining claims which involve the receptor as a whole appear to comply with the requirements of Art. 33(2), (3) PCT.

SECTION VIII----------

Claim 1 fails to define the receptor more clearly by its function (see e.g. claim 3).

As already mentioned in section V, part of the claims refer to matter not defined by a single technical feature which is clearly in contrast to the requirements of Art. 6 PCT (claims 29, 30, 40,56, 57, 63, 64, 66 and 67).

The subject-matter of claims 36-46, 60-62 and 65 is not supported by the

description (Art. 6 PCT) which is in view of the broad field covered insufficient (Art. 5 PCT). The specification lacks detailed instructions and does not even disclose one example demonstrating

a) the inhibiting and inactivating effect of the pharmaceutical composition according to claims 36-40,

b) the successful production of a transgenic non-human mammal fulfilling all the criteria as listed in claims 41 to 46 and/or,

c) the functioning of the methods of claims 60-62 and 65 which make use of the transgenic non human mammal according to claims 41 to 46.

The expression "capable of" is vague and open to interpretation.

Bxl (KDC) - 7 January 1998

P.SCRE.01/WO (B)

CLAIMS

1. Receptor which has an amino acid sequence having more than 60% homology with the amino acid sequence shown in Figure 1.

2. Receptor according to claim 1, which has the amino acid sequence shown in Figure 1.

3. Receptor according to claim 1 or 2 having a preference for pyrimidine nucleotides over purine nucleotides.

4. Receptor according to claim 3, having at least a twofold preference, preferably tenfold to one hundredfold preference for pyrimidine nucleotides over purine nucleotides.

5. Receptor according to any of the claims 3 or 4, wherein the pyrimidine nucleotide is uridine triphosphate.

6. Receptor according to any of the claims 3 to 5, having a preference for UTP over UDP.

7. Receptor according to claim 5 being a high affinity UTP-specific receptor.

8. Receptor according to any of the preceding claims, belonging to the P2 receptor family.

9. Receptor according to any of the preceding claims, being a G protein-coupled receptor.

10. Receptor according to any of the preceding claims, being a human receptor.

11. Nucleic acid molecule encoding the receptor according to any of the preceding claims.

12. Nucleic acid molecule according to claim 11, wherein the nucleic acid molecule is DNA or RNA molecule.

13. DNA molecule according to claim 12, which is a cDNA molecule or a genomic DNA molecule.

14. Nucleic acid molecule according to any of the claims 11 to 13, having more than 60% homology to the DNA sequence shown in Figure 1.

15. DNA molecule according to claim 14, which has the DNA sequence shown in figure 1.

16. Vector comprising the nucleic acid molecule according to any of the claims 11 to 15.

17. Vector according to claim 16, adapted for expression in a cell, which comprises the regulatory elements necessary for expression of the nucleic acid molecule in said cell operatively linked to the nucleic acid molecule according to any of the claims 11 to 15 as to permit expression thereof.

18. Vector of claim 17, wherein the cell is chosen among the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells.

19. Vector according to any of the claims 16 to 18, wherein the vector is a plasmid or a virus.

20. Vector according to claim 19, being a virus selected from the group consisting of baculovirus, adenovirus or Semliki Forest virus.

21. Cell comprising the vector according to any of the claims 16 to 20.

22. Cell of claim 21, wherein the cell is a mammalian cell, preferably non neuronal in origin.

23. Cell of claim 21, wherein the cell is chosen among the group consisting of COS-7 cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 cells.

24. Nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridising with a unique sequence included within the nucleic acid molecule according to any of the claims 11 to 15.

25. Nucleic acid probe of claim 24, wherein the nucleic acid is DNA or RNA.

26. Antisense oligonucleotide having a sequence capable of specifically hybridising to a mRNA molecule of claim 12, so as to prevent translation of the mRNA molecule.

27. Antisense oligonucleotide having a sequence capable of specifically hybridising to the DNA molecule of claim 13.

28. Antisense oligonucleotide according to claim 26 or 27, comprising chemical analogs of nucleotides.

29. Ligand other than purine and pyridine nucleotides capable of binding to a receptor according to any of the claims 1 to 10.

30. Anti-ligand capable of competitively inhibiting the binding of the ligand according to claim 29 to the receptor according to any of the claims 1 to 10.

31. Ligand according to claim 29 which is an antibody.

32. Anti-ligand according to claim 30 which is an antibody.

33. Antibody according to claim 31 or 32, which is a monoclonal antibody.

34. Monoclonal antibody according to claim 33, directed to an epitope of the receptor according to any of the claims 1 to 10, present on the surface of a cell expressing said receptor.

35. Pharmaceutical composition comprising an amount of the oligonucleotide according to claim 26, effective to decrease activity of the receptor according to any of the claims 1 to 10 by passing through a cell membrane and binding specifically with mRNA encoding said receptor in the cell so as to prevent its translation, and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

36. Pharmaceutical composition of claim 35, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

37. Pharmaceutical composition of claim 36, wherein the substance which inactivates mRNA is a ribozyme.

38. Pharmaceutical composition according to any of the claims 35 to 37, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure.

39. Pharmaceutical composition of claim 38, wherein the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

40. Pharmaceutical composition which comprises an effective amount of the anti-ligand of claim 30, effective to block binding of a ligand to the receptor according to any of the claims 1 to 10 and a pharmaceutically acceptable carrier.

41. Transgenic non human mammal expressing the nucleic acid molecule according to any of the claims 11 to 15.

42. Transgenic non human mammal comprising a homologous recombination knockout of the native receptor according to any of the claims 1 to 10.

43. Transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid molecule according to any of the claims 11 to 15 so placed as to be transcripted into antisense mRNA which is complementary to the mRNA of claim 12 and which hybridises to said mRNA thereby reducing its translation.

44. Transgenic non human mammal according to any of the claims 41 to 43, wherein the nucleic acid according to any of the claims 11 to 15 additionally comprises an inducible promoter.

45. Transgenic non human mammal according to any of the claims 41 to 43, wherein the nucleic acid according to claim 11 to 15 additionally comprises tissue specific regulatory elements.

46. Transgenic non human mammal according to any of the claims 41 to 45, which is a mouse.

47. Method for determining whether a ligand can specifically bind to a receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting binding of ligand to such receptor and detecting the presence of any such ligand bound specifically to said receptor, thereby determining whether the ligand binds specifically to said receptor.

48. Method for determining whether a ligand can specifically bind to the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under conditions permitting binding of the ligand to such receptor and detecting the presence of any ligand bound to said receptor, thereby determining whether the compound is capable of specifically binding to said receptor.

49. Method for determining whether a ligand is an agonist of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting the activation of a functional receptor response from the cell and detecting by means of a bio-assay, such as a modification in a second messenger concentration or a modification in the cellular metabolism, an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

50. Method for determining whether a ligand is an agonist of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in the production of a second messenger, an increase in the

receptor activity, thereby determining whether the ligand is a receptor agonist.

51. Method for determining whether a ligand is an antagonist of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in a second messenger concentration or a modification in the cellular metabolism, a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

52. Method for determining whether a ligand is an antagonist of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in a second messenger concentration, a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

53. A method according to any of the claims 47 to 50, wherein the second messenger assay comprises measurement of intra-cellular cAMP, intra-cellular Inositol phosphate, intra-cellular diacylglycerol concentration or intra-cellular calcium mobilisation.

54. Method according to any of the claims 47 to 53, wherein the cell is a mammalian cell, preferably non neuronal in origin, and chosen among the group consisting of COS-7 cells, CHO cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 cells.

55. Method according to any of the claims 47 to 54, wherein the ligand is not previously known.

56. Ligand detected by the method according to any of the preceding claims 47 to 55.

57. Pharmaceutical composition which comprises the ligand according to claim 56 and a pharmaceutically acceptable carrier.

58. Method of detecting the expression of the receptor according to any of the claims 1 to 10, by detecting the presence of mRNA coding said receptor, which comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to claim 23 under hybridising conditions, and detecting the presence of mRNA hybridised to the probe, thereby detecting the expression of the receptor by the cell.

59. Method of detecting the presence of the receptor according to any of the claims 1 to 10 on the surface of a cell, which comprises contacting the cell with the antibody of claim 31 under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of the receptor on the surface of the cell.

60. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 10, which comprises

producing a transgenic non human mammal according to any of the claims 41 to 46 whose levels of receptor expression are varied by use of an inducible promoter which regulates the receptor expression.

61. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 10, which comprises producing a panel of transgenic non human mammals according to any of the claims 41 to 46, each expressing a different amount of said receptor.

62. Method for identifying an antagonist of the receptor according to any of the claims 1 to 10 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of the receptor, which comprises administering the antagonist to a transgenic non human mammal according to any of the claims 41 to 46 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the transgenic non human mammal as a result of receptor activity, thereby identifying the antagonist.

63. Antagonist identified by the method of claim 62.

64. Pharmaceutical composition comprising an antagonist according to claim 63 and a pharmaceutically acceptable carrier.

65. Method for identifying an agonist of the receptor according to any of the claims 1 to 10 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by activation of said receptor, which comprises administering the agonist to a transgenic non human mammal according to any of the claims 41 to 46 and determining whether the antagonist alleviates the

physical and behavioural abnormalities displayed by the transgenic non human mammal, the alleviation of the abnormalities indicating the identification of the agonist.

66. Agonist identified by the method of claim 65.

67. Pharmaceutical composition comprising an agonist according to claim 66 and a pharmaceutically acceptable carrier.

68. Method for diagnosing a predisposition to a disorder associated with the activity of a specific allele of the receptor according to any of the claims 1 to 10, which comprises :

a) obtaining nucleic acid molecules of subjects suffering from said disorder;

b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;

c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;

d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridising to said nucleic acid molecule and labelled with a detectable marker;

e) detecting labelled bands which have hybridised to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;

f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and

g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose

thereby predisposition to the disorder if the patterns are the same.

59. Method of preparing the purified receptor according to any of the claims 1 to 10, which comprises :

a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b) inserting the vector of step a in a suitable host cell;

c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;

d) recovering the receptor so obtained; and

e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A1

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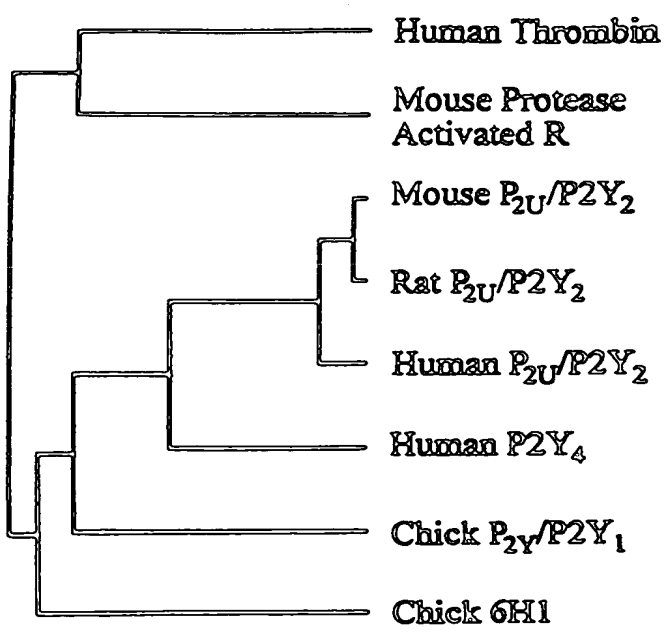
(54) Title: RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR

(57) Abstract

The present invention concerns a new receptor having a preference for pyrimidine nucleotides, preferably UTP, over purine nucleotides, and which has an amino acid sequence having more than 60 % homology with the amino acid sequence shown in Figure 1.



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RECEPTOR AND NUCLEIC ACID MOLECULE ENCODING SAID RECEPTOR.

Object of the present invention.

The present invention concerns a new receptor having a preference for pyrimidine nucleotides preferably uridine triphopshate over purine nucleotides and the nucleic acid molecule encoding said receptor, vectors comprising said nucleic acid molecule, cells transformed by said vector, antibodies directed against said receptor, nucleic acid probes directed against said nucleic acid molecule, pharmaceutical compositions comprising said products and non human transgenic animals expressing the receptor according to the invention or the nucleic acid molecule according to said receptor.

The invention further provides methods for determining ligand binding, detecting expression, screening for drugs, molecular binding specifically to said receptor and treatment involving the receptor according to the invention.

Background of the invention.

The cloning of several receptors for ATP has been reported since 1993. In keeping with the latest nomenclature proposal, these P2 purinergic receptors can be subdivided

into two classes: G protein-coupled receptors, or P2Y receptors, and receptors with intrinsic ion channel activity or P2X receptors (2). Two distinct rat P2X receptors have been cloned, respectively from the vas deferens (3) and phaechromocytoma PC12 cells (4): they have a characteristic topology, with two hydrophobic putatively membrane-spanning segments and an ion pore motif reminiscent of potassium channels. In the P2Y family, the sequences of two subtypes, both coupled to phospholipase C, have been published: chick (5), turkey (6), bovine (7), mouse and rat (8) P2Y1 receptors (formerly called P2Y); murine (9,10), rat (11) and human (12) P2Y2 receptors (previously named P2U) on the other hand. In addition, a P2Y3 receptor, with a preference for ADP over ATP, has been cloned from chick brain, but its sequence is not yet published (13). Furthermore, the 6H1 orphan receptor, cloned from activated chicken T lymphocytes, exhibits a significant degree of homology to the P2Y1 and P2Y2 receptors, suggesting that it also belongs to the P2Y family, although its responsiveness to nucleotides has not yet been demonstrated (14).

Summary of the invention.

This invention provides a receptor having a preference for pyrimidine nucleotides preferably uridine triphopshate over purine nucleotides. A receptor having a preference for pyrimidine nucleotides over purine nucleotides means a receptor for which pyrimidine nucleotides and purine nucleotides are not equally active and equipotent. This means that the receptor according to the invention in presence of these agonists presents a functional response (preferably the accumulation of Inositol triphosphate (IP3), diacylglycerol (DAG), or calcium ions) to lower concentration of pyrimidine nucleotides, preferably uridine triphopshate, than to purine

nucleotides or a more important functional response to similar concentration of pyrimidine nucleotide than to purine nucleotide.

The inositol phosphate (IP3) accumulation after addition of said agonists is described in the specification thereafter.

Advantageously, the receptor according to the invention has at least a twofold, preferably a tenfold to one hundredfold preference for pyrimidine nucleotides over purine nucleotides.

A preferred embodiment of the receptor according to the invention is characterized by a preference for uridine triphosphate over adenine nucleotides.

The receptor according to the invention is a receptor, preferably a G protein-coupled receptor, which belongs structurally to the purinergic receptor family (P2Y family) but functionally is a pyrimidinergic receptor, preferably a UTP-specific receptor.

According to a preferred embodiment of the present invention, the receptor is a human receptor.

Said receptor has an amino acid sequence having more than 60% homology with the amino acid sequence shown in figure 1. Preferably, the amino acid sequence of the receptor according to the invention has at least the amino acid sequence shown in figure 1 or a portion thereof.

A portion of the amino acid sequence means a peptide or a protein having the same binding properties as the receptor according to the invention (i.e. peptide or a protein which is characterized by a preference for pyrimidine nucleotides, preferably UTP, over purine nucleotides).

The present invention is also related to a nucleic acid molecule, such as a DNA molecule or an RNA molecule,

encoding the receptor according to the invention.

Preferably, said DNA molecule is a cDNA molecule or a genomic DNA molecule.

Preferably, said nucleic acid molecule has more than 60% homology to the DNA sequence shown in figure 1.

Preferably, the nucleic acid molecule according to the invention is at least the DNA sequence shown in figure 1 or portion thereof. "A portion of a nucleic acid sequence" means a nucleic acid sequence encoding at least a portion of amino acid sequence as described above.

The present invention is also related to a vector comprising the nucleic acid molecule according to the invention. Preferably, said vector is adapted for expression in a cell and comprises the regulatory elements necessary for expressing the amino acid molecule in said cell operatively linked to the nucleic acid sequence according to the invention as to permit expression thereof.

Preferably, said cell is chosen among the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells. The vector according to the invention is a plasmid or a virus, preferably a baculovirus, an adenovirus or a semliki forest virus.

The plasmid may be the pcDNA3-P2Y4.

The present invention concerns also the cell (preferably a mammalian cell, such as a 1321N1 cell) transformed by the vector according to the invention. Advantageously, said cell is preferably non neuronal in origin and is chosen among the group consisting of a COS-7 cell, an LM(tk-) cell, an NIH-3T3 cell or a 1321N1 cell.

The present invention is also related to a nucleic acid probe comprising the nucleic acid molecule according to the invention, of at least 15 nucleotides capable of

specifically hybridizing with a unique sequence included in the sequence of the nucleic acid molecule encoding the receptor according to the invention. Said nucleic acid probe may be a DNA or an RNA molecule.

The invention concerns also an antisense oligonucleotide having a sequence capable of specifically hybridizing to an mRNA molecule encoding the receptor according to the invention so as to prevent translation of said mRNA molecule or an antisense oligonucleotide having a sequence capable of specifically hybridizing to the cDNA molecule encoding the receptor according to the invention.

Said antisense oligonucleotide may comprise chemical analogs of nucleotide or substances which inactivate mRNA, or be included in an RNA molecule endowed with ribozyne activity.

Another aspect of the present invention concerns a ligand other than purine and pyrimidine nucleotides (preferably an antibody) capable of binding to a receptor according to the invention and an anti-ligand (preferably also an antibody) capable of competitively inhibiting the binding of said ligand to the receptor according to the invention.

Preferably, said antibody is a monoclonal antibody.

The present invention concerns also the monoclonal antibody directed to an epitope of the receptor according to the invention and present on the surface of a cell expressing said receptor.

The invention concerns also the pharmaceutical composition comprising an effective amount of oligonucleotide according to the invention, effective to decrease the activity of said receptor by passing through a cell membrane and binding specifically with mRNA encoding the receptor

according to the invention in the cell so as to prevent its translation. The pharmaceutical composition comprises also a pharmaceutically acceptable carrier capable of passing through said cell membrane.

Preferably, in said pharmaceutical composition, the oligonucleotide is coupled to a substance, such as a ribozyme, which inactivates mRNA.

Preferably, the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure. The structure of the pharmaceutically acceptable carrier in said pharmaceutical composition is capable of binding to a receptor which is specific for a selected cell type.

Preferably, said pharmaceutical composition comprises an amount of the antibody according to the invention effective to block the binding of a ligand to the receptor according to the invention and a pharmaceutically acceptable carrier.

The present invention concerns also a transgenic non human mammal overexpressing (or expressing ectopically) the nucleic acid molecule encoding the receptor according to the invention.

The present invention also concerns a transgenic non human mammal comprising a homologous recombination knockout of the native receptor according to the invention.

According to a preferred embodiment of the invention, the transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid according to the invention is so placed as to be transcripted into antisense mRNA which is complementary to the mRNA encoding the receptor according to the invention and

which hybridizes to mRNA encoding said receptor, thereby reducing its translation. Preferably, the transgenic non human mammal according to the invention comprises a nucleic acid molecule encoding the receptor according to the invention and comprises additionally an inducible promoter or a tissue specific regulatory element.

Preferably, the transgenic non human mammal is a mouse.

The invention relates to a method for determining whether a ligand can be specifically bound to the receptor according to the invention, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting binding of ligand to such receptor and detecting the presence of any such ligand bound specifically to said receptor, thereby determining whether the ligand binds specifically to said receptor.

The invention relates to a method for determining whether a ligand can specifically bind to a receptor according to the invention, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under conditions permitting binding of the ligand to such receptor and detecting the presence of any ligand bound to said receptor, thereby determining whether the compound is capable of specifically binding to said receptor. Preferably, said method is used when the ligand is not previously known.

The invention relates to a method for determining whether a ligand is an agonist of the receptor according to the invention, which comprises contacting a cell transfected

with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting the activation of a functional receptor response from the cell and detecting by means of a bio-assay, such as a modification in a second messenger concentration or a modification in the cellular metabolism (preferably determined by the acidification rate of the culture medium), an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

The invention relates to a method for determining whether a ligand is an agonist of the receptor according to the invention, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in the production of a second messenger an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

The present invention relates to a method for determining whether a ligand is an antagonist of the receptor according to the invention, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in second messenger concentration or a modification in the cellular metabolism, (preferably determined by the acidification rate of the culture medium) a decrease in the

receptor activity, thereby determining whether the ligand is a receptor antagonist.

The present invention relates to a method for determining whether a ligand is an antagonist of the receptor according to the invention, which comprises preparing a cell extract from cells transfected with an expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in the production of a second messenger, a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

Preferably, the second messenger assay comprises measurement of intracellular cAMP, intracellular inositol phosphate (IP3), intracellular diacylglycerol (DAG) concentration or intracellular calcium mobilization.

Preferably, the cell used in said method is a mammalian cell non neuronal in origin, such as a COS-7 cell, a CHO cell, a LM(tk-) cell an NIH-3T3 cell or 1321N1.

In said method, the ligand is not previously known.

The invention is also related to the ligand isolated and detected by any of the preceding methods.

The present invention concerns also the pharmaceutical composition which comprises an effective amount of an agonist or an antagonist of the receptor according to the invention, effective to reduce the activity of said receptor and a pharmaceutically acceptable carrier.

For instance, said agonist or antagonist may be used in a pharmaceutical composition in the treatment of cystic fibrosis, and the method according to the invention

may be advantageously used in the detection of improved drugs which are used in the treatment of cystic fibrosis.

Therefore, the previously described methods may be used for the screening of drugs to identify drugs which specifically bind to the receptor according to the invention.

The invention is also related to the drugs isolated and detected by any of these methods.

The present invention concerns also a pharmaceutical composition comprising said drugs and a pharmaceutically acceptable carrier.

The invention is also related to a method of detecting expression of a receptor according to the invention by detecting the presence of mRNA coding for a receptor, which comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to the invention under hybridizing conditions and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of the receptor by the cell.

Said hybridization conditions are stringent conditions.

The present invention concerns also the use of the pharmaceutical composition according to the invention for the treatment and/or prevention of cystic fibrosis.

The present invention concerns also a method for diagnosing a predisposition to a disorder associated with the activity of the receptor according to the invention. Said method comprises:

a) obtaining nucleic acid molecules of subjects suffering from said disorder;

b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;

c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;

d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to said nucleic acid molecule and labelled with a detectable marker;

e) detecting labelled bands which have hybridized to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;

f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and

g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

A last aspect of the present invention concerns a method of preparing the receptor according to the invention, which comprises:

a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b) inserting the vector of step a in a suitable host cell;

c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;

d) recovering the receptor so obtained; and

e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.

Short description of the drawings.

Figure 1 represents nucleotide and deduced amino acid sequence of a human $P2Y_4$ receptor according to the invention. The putative membrane-spanning domains are underlined and numbered I to VII. The consensus sequence conserved between all the P2Y receptors and the three amino acids (AHN) corresponding to the RGD sequence in the first extracellular loop of the $P2Y_2$ receptor are represented in bold. The putative phosphorylation sites by PKC or by calmodulin-dependent protein kinases and PKC are indicated respectively by black squares (■) and by open circles (O).

Figure 2 is a dendrogram representing structural relatedness among the cloned P2Y receptor and the closest neighbour in the G protein-coupled receptor family. The plot was constructed using the multiple sequence alignment program Pileup of the GCG package (26). For each sequence, the analysis takes into account a segment covering the first five putative membrane-spanning domains.

Figure 3 represents a northern blot analysis of $P2Y_4$ receptor expression. The Northern blot was performed with 15 μg of total RNA from human placenta and 4 μg of poly(A)+ RNA from K562 cells and from two different human placentas. The probe was a human $P2Y_4$ gene fragment amplified by PCR (TM2 to TM7).

Figure 4 represents the time course of $InsP_3$ accumulation in 1321N1 cells expressing the human $P2Y_4$ receptor. ^3H inositol labelled cells were incubated for the indicated time with UTP (100 μM), UDP (100μM) and ATP (100 μM) in the absence of 10 mM LiCl (panel A) or in its presence (panel B). The data represent the mean of triplicate experimental points and are representative of two independent experiments.

Figure 5 Represents the effect of ATP on the accumulation of $InsP_3$ induced by UTP in 1321N1 transfected cells. Concentration-action curves of ATP in the presence of UTP 10 or 100 μM at 30 s (panel A) and 20 min (panel B). Concentration-action curve of ATP with or without UTP (10 μM) at 20 min (panel C). The data represent the mean ± S.D. of triplicate experimental points and are representative of two (panel A), five (panel B) or three (panel C) independent experiments.

Figure 6 represents the concentration-action curves of UTP and UDP on the $InsP_3$ accumulation in three different clones of 1321N1 transfected cells. The cells were incubated in the presence of various UTP (●) and UDP (▣) concentrations (0, 0.1, 1, 3, 10 and 100 μM) for 30 s or 20 min. The data represent the mean ± S.D. of triplicate experimental points obtained in one representative experiment. The EC_{50} values were determined by curve fitting (Sigma Plot: version 2.0).

Figure 7 Represents the effect of various nucleotides on the $InsP_3$ production in 1321N1 transfected cells.

The cells were incubated with UTP, UDP, 5BrUTP, dUTP, ITP, AP_3A, AP_4A, AP_5A and AP_6A at the same concentration of 100 μM or without agonist (Cont) for 30 s or 20 min. The data represent the mean ± S.D. of triplicate experimental points and are representative of three independent experiments. The EC_{50} values were determined by curve fitting (Sigma Plot: version 2.0).

Figure 8 Represents concentration-action curves of various nucleotides on the $InsP_3$ accumulation in 1321N1 cells expressing a human $P2Y_4$ receptor. 1321N1 cells were incubated in the presence of various concentrations of UTP, UDP, dUTP, 5BrUTP, ITP and ATP for a period of time of 20 min. The data are the mean ± range of duplicate experimental points obtained in an experiment representative of two.

Figure 9 Represents the action of various P_2 antagonists on the $InsP_3$ production induced by UTP in 1321N1 transfected cells. Cells were incubated in the presence of suramin, reactive blue 2 and PPADS at a concentration of 100 μM and different UTP concentrations (0, 2 and 10 μM) for 20 min. The data represent the mean ± S.D. of triplicate experimental points and are representative of two independent experiments.

Figure 10 Represents the effect of PPADS on the UTP stimulation of $InsP_3$ in 1321N1 transfected cells. The cells were exposed to various concentrations of UTP in the presence or in the absence of PPADS (100 μM) for 20 min. The data are the mean ± S.D. of triplicate experimental points obtained in an

experiment representative of two.

Figure 11 Represents the effect of pertussis toxin on the UTP-induced accumulation of $InsP_3$ in 1321N1 cells expressing a human $P2Y_4$ receptor. The cells were preincubated for 18 hours in the presence or in the absence of 20 ng/ml pertussis toxin. The cells were then incubated with or without UTP 100 μM and with or without pertussis toxin (20 ng/ml) for various times: 30 s, 5 min or 20 min. The data represent the mean ± S.D. of triplicate experimental points and are representative of two independent experiments.

Detailed description of the invention.

EXPERIMENTAL PROCEDURES

1. Materials

Trypsin was from Flow Laboratories (Bioggio, Switzerland) and the culture media, reagents, G418, fetal calf serum (FCS), restriction enzymes and Taq polymerase were purchased from GIBCO BRL (Grand Island, NY). The radioactive products myo-D-[2-^{3}H]inositol (17.7 Ci/mmol) and [a^{32}P]ATP (800 Ci/mmol) were from Amersham (Gent, Belgium). Dowex AG1X8 (formate form) was from Bio-Rad Laboratories (Richmond, Calif.). UTP, UDP, ATP, ADP, carbachol, LiCl and apyrase grade VII were obtained from Sigma Chemical Co. (St. Louis, MO). 2MeSATP was from Research Biochemicals Inc. (Natick, MA). pcDNA3 is an expression vector developed by Invitrogen (San Diego, CA).

2. Cloning and sequencing

Degenerate oligonucleotide primers were synthesized on the basis of the best conserved segments between the murine P2Y2 and the chick P2Y1 receptor sequences. These primers were used to amplify novel receptor gene fragments

by low-stringency PCR starting from human genomic DNA. The amplification conditions were as follows: 93 °C 1 min, 50 °C 2 min, 72 °C 3 min; 35 cycles. The PCR products with sizes compatible with P2 receptor gene fragments were subcloned in M13mp18 and M13mp19 and sequenced by the Sanger dideoxy nucleotide chain termination method. One of the resulting clones sharing similarities with P2 receptors, was labelled by random priming and used to screen a human genomic DNA library constructed in the λ Charon 4a vector. The hybridization was in 6 x SSC (1 x SSC: 0.15 M NaCl, 0.015 M Sodium citrate) and 40% formamide at 42 °C for 14 h and the final wash conditions were 0.1 x SSC, 0.1% SDS at 65 °C A preparation of λ phages (15) was made for several clones which hybridized strongly with the probe. A restriction map and a Southern blotting analysis allowed to isolate a 1.4 kb NheI-EcoRV fragment that was subcloned into the pBluescript SK⁺ vector (Stratagene). The complete sequence of a new receptor coding sequence was obtained on both strands after subcloning of overlapping fragments in M13mp18 and M13mp19.

3. Cell culture and transfection

The $P2Y_4$ receptor coding sequence was subcloned between the HindIII and the EcoRV sites of the pcDNA3 expression vector for transfection into 1321N1 human astrocytoma cells, a cell line which does not respond to nucleotides and which has already been used for the expression of purinergic receptors (6,12). Cells were transfected with the recombinant pcDNA3 plasmid (pcDNA3-$P2Y_4$) using the calcium phosphate precipitation method as described (16). 1321N1 cells were incubated for 6 hours at 37 °C in the presence of pcDNA3 vector alone or vector containing the $P2Y_4$ receptor coding sequence, then washed and incubated in

culture medium (10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and 2.5 μg/ml amphotericin B in Dulbecco's modified Eagle's medium (DMEM)). The selection with G418 (400 μg/ml) was started two days after transfection. From the pool of transfected 1321N1 cells, individual clones were isolated by limiting dilution with the aim of selecting clones with high IP stimulation factors in response to nucleotides. The different clones were maintained in a medium containing 400 μg/ml G418.

4. Inositol phosphates (IP) measurement

1321N1 cells were labelled for 24 hours with 10 μCi/ml [^{3}H] inositol in inositol-free DMEM (Dulbecco's modified Eagle's medium) medium containing 5% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin B and 400 μg/ml G418. Cells were washed twice with KRH (Krebs-Ringer Hepes) buffer of the following composition (124 mM NaCl, 5 mM KCl, 1.25 mM $MgSO_4$, 1.45 mM $CaCl_2$, 25 mM Hepes (pH 7.4) and 8 mM glucose) and incubated in this medium for 30 min . The agonists were added in the presence of LiCl (10 mM) and the incubation was stopped after 30 s, 5 min or 20 min by the addition of an ice-cold 3% perchloric acid solution. For the time course study, LiCl (10 mM) was added 5 min before the agonists and the incubation was stopped at different times. When tested, pertussis toxin (20 ng/ml) was added for 18 h during the labelling period time and during the stimulation by the agonist. Inositol phosphates were extracted and $InsP_3$ was isolated by chromatography on Dowex column as described previously (17).

5. Radioligand binding assay.

Binding assays of [α^{32}P] UTP to cell membranes were carried out in Tris-HCl (50 mM, pH 7.5), EDTA 1 mM in a final volume of 0.5 ml, containing 25-50 μg of protein and 0.5 nM

of radioligand (27). The assays were conducted at 30°C for 5 min. Incubations were stopped by the addition of 4 ml of ice-cold Tris-HCl (50 mM, pH 7.5) and rapid filtration through Whatman GF/B filters under reduced pressure. The filters were then washed three times with 2 ml of the same ice-cold Tris-HCl buffer. Radioactivity was quantified by liquid scintillation counting, after an overnight incubation of the filters in liquid scintillation mixture.

6. Northern blot and Southern blot analysis

Total and poly(A)$^+$ RNA were prepared from different tissues and human cell lines using the guanidinium thiocyanate-cesium chloride procedure (15), denatured by glyoxal and fractionated by electrophoresis on a 1% agarose gel in 10 mM phosphate buffer pH 7.0. DNA samples, prepared from the λ Charon 4a clones, were digested with restriction enzymes. Northern and Southern blots were prepared (15) and baked for 90 min at 80 °C. Membranes were prehybridized for at least 4 hours and hybridized overnight with the same probe as for the screening, at 42 °C in a solution containing 50% formamide for Northern blots and 40% formamide for Southern blots. Filters were washed twice for 15 min in 2 x SSC at room temperature and then twice for 30 min in 0.2 x SSC at 60 °C before being exposed at -70 °C in the presence of intensifying screens for 5 days (Northern blots) or 1 hour (Southern blots).

RESULTS

1. Cloning and sequencing

In order to isolate new subtypes of P2 receptors, sets of degenerate oligonucleotide primers were synthesized on the basis of the best conserved segments in the published sequences of the chick brain P2Y1 (5) and murine

neuroblastoma P2Y2 (9) receptors. These primers were used in low-stringency PCR on human genomic DNA as described (18). Some combinations generated discrete bands with a size compatible with that expected for P2 receptors. For example, the primer [5'-CAGATCTAGATA(CT)ATGTT(CT)(AC)A(CT)(CT)T(ACGT)GC-3'] corresponding to the second transmembrane region and the primer [5'-TCTTAAGCTTGG(AG)TC(ACGT)A(CG)(AG)CA(AG)CT(AG)TT-3'] corresponding to the seventh transmembrane region amplified a 712 bp fragment. The partial sequences obtained after sequencing were translated into peptidic sequences and compared to a local databank which contains G protein-coupled receptor sequences. Most of the clones resulting from these PCR products encoded a part of a new receptor which displayed 58% identity with the murine P2Y2 receptor and 42% identity with the chick P2Y1 receptor partial sequences. In addition, some clones encoded a peptidic sequence presenting 87% identity with the chick P2Y1 receptor and are therefore believed to represent fragments of the human P2Y1 gene.

The partial sequence of the new receptor was used as a probe to screen a human genomic DNA library. Several clones that strongly hybridized with the probe at high stringency conditions were obtained and purified. The inserts of the clones varied from 12 to 17 kb and restriction analysis revealed that all clones belonged to a single locus. The full sequence of a 1.4 kb NheI-EcoRV fragment was obtained and an intronless open reading frame of 1095 bp was identified. The sequence is depicted in figure 1 where the putative membrane-spanning domains are underlined and numbered I to VII. The predicted molecular weight of the encoded protein is 36.5 kDa. This molecular weight is unlikely to be modified in vivo, since no N-glycosylation consensus sequences are found in the putative exofacial

regions. In contrast with the human P2Y2 receptor, there is no RGD motif, an integrin binding consensus sequence, in the putative first extracellular loop. The three amino acid (AHN) corresponding to the RGD sequence in the first extracellular loop of the P2Y2 receptor are represented in bold in figure 1. Some potential sites of phosphorylation by protein kinase C (PKC) or by calmodulin-dependent protein kinases were identified in the third intracellular loop and in the carboxyterminal part of the receptor. The putative phosphorylation sites by PKC or by calmodulin-dependent protein kinases and PKC are indicated respectively by black squares and by open circles in figure 1. The four positively charged amino acid which have been reported to play a role in the P2Y2 receptor activation by ATP and UTP (1) are conserved in the P2Y4 sequence: His^{262}, Arg^{265}, Lys^{289} and Arg^{292} (Figure 1). The P2Y4 amino acid sequence was compared to the chick P2Y1 and the murine P2Y2 amino acid sequences and to their closest neighbours in the G protein-coupled receptor family (Figure 2). The plot was constructed using the multiple sequence alignment program Pileup of the GCG package (26). For each sequence, the analysis takes into account a segment covering the first five putative membrane-spanning domains. It is clear that, from a structural point of view, the newly cloned receptor is more closely related to the human P2Y2 receptor (51% of identity between the complete sequences) than to the chick P2Y1 receptor (35%).

2. Tissue distribution of the P2Y4 receptor

The tissue distribution of P2Y4 transcripts was investigated by Northern blotting. A number of rat tissues (heart, brain, liver, testis and kidney) were tested using a human probe at low stringency, but no hybridization signal could be obtained. No P2Y4 transcript could be detected in

the following human cell lines: K562 leukemia cells (Figure 3), HL-60 leukemia cells and SH-SY5Y human neuroblastoma cells. The Northern blot was performed with 15 μg of total RNA from human placenta and 4 μg of poly(A)+ RNA from K562 cells and from two different human placentas. The probe was the human P2Y4 gene fragment amplified by PCR (TM2 to TM7). On the contrary, a strong signal, corresponding to a 1.8 kb mRNA, was found in human placenta (Figure 3).

3. Functional expression of the new P2 receptor in 1321N1 cells

After transfection of the pcDNA3-P2Y4 construction in 1321N1 cells, the pool of G418-resistant clones was tested for their functional response (IP3 accumulation) to ATP and UTP. Both nucleotides were found to be agonists of the P2Y4 receptor, but the response to UTP was more robust. About 20 transfected clones were then isolated and tested for their response to UTP. The clone presenting the highest IP accumulation factor in response to UTP was selected and used in all subsequent experiments. Functional characterization of the $P2Y_4$ receptor was performed by determining the accumulation of $InsP_3$ after 20 min incubation with the agonists in the presence of 10 mM LiCl. We observed that the response to UTP was biphasic, with a peak reached at 30 s, followed by a more sustained stimulation of lower magnitude (Fig. 4A). With ATP, only that second phase was detectable: its effect became apparent after 1 min of stimulation only and was stable for at least 20 min (Fig. 4A and B). As for UTP, the stimulation by UDP was biphasic, but it was slightly delayed (Fig 4A and B). Inclusion of LiCl had little effect on the initial peak induced by UTP or UDP, but it strongly enhanced the following plateau phase (Fig. 4B).

The maximal effect of ATP observed after a 20 min

incubation represented about 27 ± 9% of that of UTP (mean ± S.D. of ten experiments). In order to demonstrate that ATP is able to antagonize the UTP response, incubations of 1321N1 cells were conducted with ATP alone or in combination with UTP. Figure 5 shows that at high concentration (500 μM or more), ATP was able to inhibit the effect of UTP, both at 30 s and 20 min. At 30 s, the response to UTP 10 μM was fully antagonized by ATP 2 mM, corresponding to the fact that ATP has no effect on the human $P2Y_4$ receptor at this early time (panel A). At 20 min, an inhibition of 62 ± 11% of the UTP effect (10 μM), corresponding to the difference between the UTP and the ATP effects, was observed in the presence of 2 mM ATP (mean ± S.D. of five independent experiments) (panels B and C). The ATP concentration-inhibition curves were shifted to the right when the UTP concentration was increased, indicating the competitive nature of this inhibitory effect (panels A and B). On the other hand, at lower concentrations (30-300 μM), ATP enhanced the response to UTP by 29% (range 12-47%, mean of four experiments) (panel B). ADP, which had almost no effect per se and did not inhibit the action of UTP, reproduced that enhancement: in the presence of ADP (100 μM), the stimulation by UTP (10 μM) represented 158 ± 15% (mean of three independent experiments) of that by UTP alone (data not shown). However, this potentiating effect of ATP and ADP was not specific: indeed the action of carbachol mediated by muscarinic receptors endogenously expressed in the 1321N1 cells (6) was also increased in the presence of these nucleotides. This observation was reproduced with cells transfected with the recombinant $P2Y_4$-pcDNA3 plasmid or with the vector alone and was also obtained with AMP and adenosine (data not shown).

We compared the concentration-action curves of UTP

and UDP on the $InsP_3$ production for several clones of transfected cells. The study was made at two times (Fig. 6) : 30 s and 20 min. In the set of experiments performed on clone 11 (clone of 1321N1 transfected cells chosen for the pharmacological characterization), UTP appeared to be 10-fold more potent than UDP after a 20 min incubation and this difference was reproduced with two other clones (Fig. 6). The EC_{50} values were 0.3 ± 0.1 μM and 3.3 ± 0.6 μM in clone 2, 2.4 ± 0.1 μM and 19.8 ± 4.8 μM in clone 11 and 0.3 ± 0.1 μM and 3.2 ± 0.8 μM in clone 21, respectively, for UTP and UDP (mean ± S.D. of two independent experiments). At 30 s of incubation, it was not possible to determine EC_{50} values because the curves were clearly shifted to the right, but we can observe that the difference between the two agonists potency was even more striking (Fig. 6). Several clones, including clones 2, 11 and 21 were tested in binding studies with [α^{32}P] UTP but no increase in specific binding was observed as compared to the cells transfected with the vector alone (data not shown).

In view of the time differences observed in Figure 6, the testing of a range of nucleotides was performed at two times: 30 s and 20 min. As Figure 7 shows, several agonists were barely or not active at 30 s (UDP, 5BrUTP, dUTP, ITP) whereas they produced a significant effect at 20 min. Full concentration-action curves were obtained at 20 min. The rank order of potency was: UTP>UDP=dUTP>5BrUTP>ITP>ATP (Fig. 8). The EC_{50} values obtained were the following: EC_{50} UTP = 2.5 ± 0.6 μM, EC_{50} UDP = 19.5 ± 3.9 μM (mean ± S.D. of eight independent experiments), EC_{50} dUTP = 20.0 ± 2.3 μM, EC_{50} 5BrUTP = 27.1 ± 1.9 μM and EC_{50} ITP = 32.8 ± 5.4 μM (mean ± S.D. of two independent experiments). The approximative EC_{50} value obtained for ATP was: 43 ± 12 μM (mean ± S.D. of five

independent experiments). The diadenosine polyphosphates also increased the $InsP_3$ production in transfected cells with EC_{50} between 3 and 7 μM (data not shown), but their maximal effect was only 20-25 % of that of UTP, a value close to that of ATP (range of four independent experiments) (Fig. 7). UMP, uridine, AMP, adenosine and ATPγS were without any effect (data not shown).

No specific antagonist is available for any P2Y subtype. Nonetheless, several non-selective antagonists such as suramin, RB2 or PPADS have been tested on P_2 receptors and their relative actions on these subtypes may constitute a mean to discriminate them (27). So we tested the ability of these three antagonists to inhibit the UTP response in the model of the human $P2Y_4$ receptor. As we can see on figure 9, PPADS appeared to be the most active antagonist (73 ± 14% inhibition; IC_{50} around 15 μM (data not shown)), suramin was inactive, and RB-2 produced an inhibition of 33 ± 5% of the UTP response (mean ± S.D. of two independent experiments). Figure 10 shows the mixed nature of the antagonism by PPADS of the UTP response: it affects both the EC_{50} value and the maximal effect of UTP. The EC_{50} value for UTP in the absence of PPADS was 3.3 ± 0.6 μM and 12.2 ± 4.5 μM in the presence of 100 μM PPADS (mean ± S.D. of two independent experiments).

The effect of pertussis toxin (20 ng/ml, 18 hours pretreatment) was studied at different times after UTP (100 μM) addition (Fig. 11). The UTP response was clearly inhibited at 30 s (62 ± 5% of inhibition: mean ± S.D. of two independent experiments), whereas no significant effect was observed at 5 and 20 min.

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CLAIMS.

1. Receptor which has an amino acid sequence having more than 60% homology with the amino acid sequence shown in Figure 1.

2. Receptor according to claim 1, which has at least the amino acid sequence shown in Figure 1 or a portion thereof.

3. Receptor according to claim 1 or 2 having a preference for pyrimidine nucleotides over purine nucleotides.

4. Receptor according to claim 3, having at least a twofold preference, preferably tenfold to one hundredfold preference for pyrimidine nucleotides over purine nucleotides.

5. Receptor according to any of the claims 3 or 4, wherein the pyrimidine nucleotide is uridine triphosphate.

6. Receptor according to any of the claims 3 to 5, having a preference for UTP over UDP.

7. Receptor according to claim 5 being a high affinity UTP-specific receptor.

8. Receptor according to any of the preceding claims, belonging to the P2 receptor family.

9. Receptor according to any of the preceding claims, being a G protein-coupled receptor.

10. Receptor according to any of the preceding claims, being a human receptor.

11. Nucleic acid molecule encoding the receptor according to any of the preceding claims.

12. Nucleic acid molecule according to claim 11, wherein the nucleic acid molecule is DNA or RNA molecule.

13. DNA molecule according to claim 12, which is a cDNA molecule or a genomic DNA molecule.

14. Nucleic acid molecule according to any of the claims 11 to 13, having more than 60% homology to the DNA sequence shown in Figure 1.

15. DNA molecule according to claim 14, which has at least the DNA sequence as shown in figure 1 or a portion thereof.

16. Vector comprising the nucleic acid molecule according to any of the claims 11 to 15.

17. Vector according to claim 16, adapted for expression in a cell, which comprises the regulatory elements necessary for expression of the nucleic acid molecule in said cell operatively linked to the nucleic acid molecule according to any of the claims 11 to 15 as to permit expression thereof.

18. Vector of claim 17, wherein the cell is chosen among the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells.

19. Vector according to any of the claims 16 to 18, wherein the vector is a plasmid or a virus, preferably a baculovirus, an adenovirus or a Semliki Forest virus.

20. Vector according to claim 19, wherein the plasmid is pcDNA3-P2Y4.

21. Cell comprising the vector according to any of the claims 16 to 20.

22. Cell of claim 21, wherein the cell is a mammalian cell, preferably non neuronal in origin.

23. Cell of claim 21, wherein the cell is chosen among the group consisting of COS-7 cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 cells.

24. Nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the

nucleic acid molecule according to any of the claims 11 to 15.

25. Nucleic acid probe of claim 24, wherein the nucleic acid is DNA or RNA.

26. Antisense oligonucleotide having a sequence capable of specifically hybridizing to a mRNA molecule of claim 12, so as to prevent translation of the mRNA molecule.

27. Antisense oligonucleotide having a sequence capable of specifically hybridizing to the DNA molecule of claim 13.

28. Antisense oligonucleotide according to claim 26 or 27, comprising chemical analogs of nucleotides.

29. Ligand other than purine and pyridine nucleotides capable of binding to a receptor according to any of the claims 1 to 10.

30. Anti-ligand capable of competitively inhibiting the binding of the ligand according to claim 29 to the receptor according to any of the claims 1 to 10.

31. Ligand according to claim 29 which is an antibody.

32. Anti-ligand according to claim 30 which is an antibody.

33. Antibody according to claim 31 or 32, which is a monoclonal antibody.

34. Monoclonal antibody according to claim 33, directed to an epitope of the receptor according to any of the claims 1 to 10, present on the surface of a cell expressing said receptor.

35. Pharmaceutical composition comprising an amount of the oligonucleotide according to claim 26, effective to decrease activity of the receptor according to any of the claims 1 to 10 by passing through a cell membrane

and binding specifically with mRNA encoding said receptor in the cell so as to prevent its translation, and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

36. Pharmaceutical composition of claim 35, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

37. Pharmaceutical composition of claim 36, wherein the substance which inactivates mRNA is a ribozyme.

38. Pharmaceutical composition according to any of the claims 35 to 37, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure.

39. Pharmaceutical composition of claim 38, wherein the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

40. Pharmaceutical composition which comprises an effective amount of the anti-ligand of claim 30, effective to block binding of a ligand to the receptor according to any of the claims 1 to 10 and a pharmaceutically acceptable carrier.

41. Transgenic non human mammal expressing the nucleic acid molecule according to any of the claims 11 to 15.

42. Transgenic non human mammal comprising a homologous recombination knockout of the native receptor according to any of the claims 1 to 10.

43. Transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid molecule according to any of the claims 11 to 15 so

placed as to be transcripted into antisense mRNA which is complementary to the mRNA of claim 12 and which hybridizes to said mRNA thereby reducing its translation.

44. Transgenic non human mammal according to any of the claims 41 to 43, wherein the nucleic acid according to any of the claims 11 to 15 additionally comprises an inducible promoter.

45. Transgenic non human mammal according to any of the claims 41 to 44, wherein the nucleic acid according to claim 11 to 15 additionally comprises tissue specific regulatory elements.

46. Transgenic non human mammal according to any of the claims 41 to 45, which is a mouse.

47. Method for determining whether a ligand can specifically bind to a receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting binding of ligand to such receptor and detecting the presence of any such ligand bound specifically to said receptor, thereby determining whether the ligand binds specifically to said receptor.

48. Method for determining whether a ligand can specifically bind to the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under conditions permitting binding of the ligand to such receptor and detecting the presence of any ligand bound to said receptor, thereby determining whether the compound is capable of specifically binding to said

receptor.

49. Method for determining whether a ligand is an agonist of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting the activation of a functional receptor response from the cell and detecting by means of a bio-assay, such as a modification in a second messenger concentration or a modification in the cellular metabolism, an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

50. Method for determining whether a ligand is an agonist of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in the production of a second messenger, an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

51. Method for determining whether a ligand is an antagonist of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in a second messenger concentration or a modification in the cellular metabolism,

a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

52. Method for determining whether a ligand is an antagonist of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in a second messenger concentration, a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

53. A method according to any of the claims 47 to 50, wherein the second messenger assay comprises measurement of intra-cellular cAMP, intra-cellular Inositol phosphate, intra-cellular diacylglycerol concentration or intra-cellular calcium mobilization.

54. Method according to any of the preceding claims 47 to 53, wherein the cell is a mammalian cell, preferably non neuronal in origin, and chosen among the group consisting of COS-7 cells, CHO cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 cells.

55. Method according to any of the preceding claims 47 to 54, wherein the ligand is not previously known.

56. Ligand detected by the method according to any of the preceding claims 47 to 55.

57. Pharmaceutical composition which comprises the ligand according to claim 56 and a pharmaceutically acceptable carrier.

58. Method of screening drugs to identify drugs

which specifically bind to the receptor according to any of the claims 1 to 10 on the surface of the cell, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs under conditions permitting binding of said drugs to the receptor, and determining those drugs which specifically bind to the transfected cell, thereby identifying drugs which specifically bind to the receptor.

59. Method of screening drugs to identify drugs which specifically bind to the receptor according to any of the claims 1 to 10 on the surface of the cell, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cells extract, contacting the membrane fraction with a plurality of drugs and determining those drugs which bind to the transfected cell, thereby identifying drugs which specifically bind to said receptor.

60. Method of screening drugs to identify drugs which act as agonists of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs under conditions permitting the activation of a functional receptor response, and determining those drugs which activate such receptor using a bio-assay, such as a modification in a second messenger concentration or modification in the cellular metabolism, thereby identifying drugs which act as receptor agonists.

61. Method of screening drugs to identify drugs which act as agonists of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from

cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs under conditions permitting the activation of a functional receptor response, and determining those drugs which activate such receptor using a bio-assay, such as a modification in a second messenger concentration, thereby identifying drugs which act as receptor agonists.

62. Method of screening drugs to identify drugs which act as antagonists of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response, and determining those drugs which inhibit the activation of the receptor using a bio-assay, such as a modification in a second messenger concentration or modification in the cellular metabolism, thereby identifying drugs which act as receptor antagonists.

63. Method of screening drugs to identify drugs which act as antagonists of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs in presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response, and determining those drugs which inhibit the activation of the receptor using a bio-assay, such as a modification in a second messenger

concentration, thereby identifying drugs which act as receptor antagonists.

64. Drug detected by any of the methods according to claims 58 to 63.

65. Pharmaceutical composition comprising a drug according to claim 64.

66. Method of detecting the expression of the receptor according to any of the claims 1 to 10, by detecting the presence of mRNA coding said receptor, which comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to claim 24 under hybridizing conditions, and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of the receptor by the cell.

67. Method of detecting the presence of the receptor according to any of the claims 1 to 10 on the surface of a cell, which comprises contacting the cell with the antibody of claim 31 under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of the receptor on the surface of the cell.

68. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 10, which comprises producing a transgenic non human mammal according to any of the claims 41 to 46 whose levels of receptor expression are varied by use of an inducible promoter which regulates the receptor expression.

69. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 10, which comprises producing a panel of transgenic non human mammals according

to any of the claims 41 to 46, each expressing a different amount of said receptor.

70. Method for identifying an antagonist of the receptor according to any of the claims 1 to 10 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of the receptor, which comprises administering the antagonist to a transgenic non human mammal according to any of the claims 40 to 45 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the transgenic non human mammal as a result of receptor activity, thereby identifying the antagonist.

71. Antagonist identified by the method of claim 70.

72. Pharmaceutical composition comprising an antagonist according to claim 71 and a pharmaceutically acceptable carrier.

73. Method for identifying an agonist of the receptor according to any of the claims 1 to 10 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by activation of said receptor, which comprises administering the agonist to a transgenic non human mammal according to any of the claims 41 to 46 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the transgenic non human mammal, the alleviation of the abnormalities indicating the identification of the agonist.

74. Agonist identified by the method of claim 73.

75. Pharmaceutical composition comprising an agonist according to claim 74 and a pharmaceutically acceptable carrier.

76. Method for diagnosing a predisposition to a

disorder associated with the activity of a specific allele of the receptor according to any of the claims 1 to 10, which comprises:

a) obtaining nucleic acid molecules of subjects suffering from said disorder;

b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;

c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;

d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to said nucleic acid molecule and labelled with a detectable marker;

e) detecting labelled bands which have hybridized to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;

f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and

g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

77. Method of preparing the purified receptor according to any of the claims 1 to 10, which comprises:

a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the

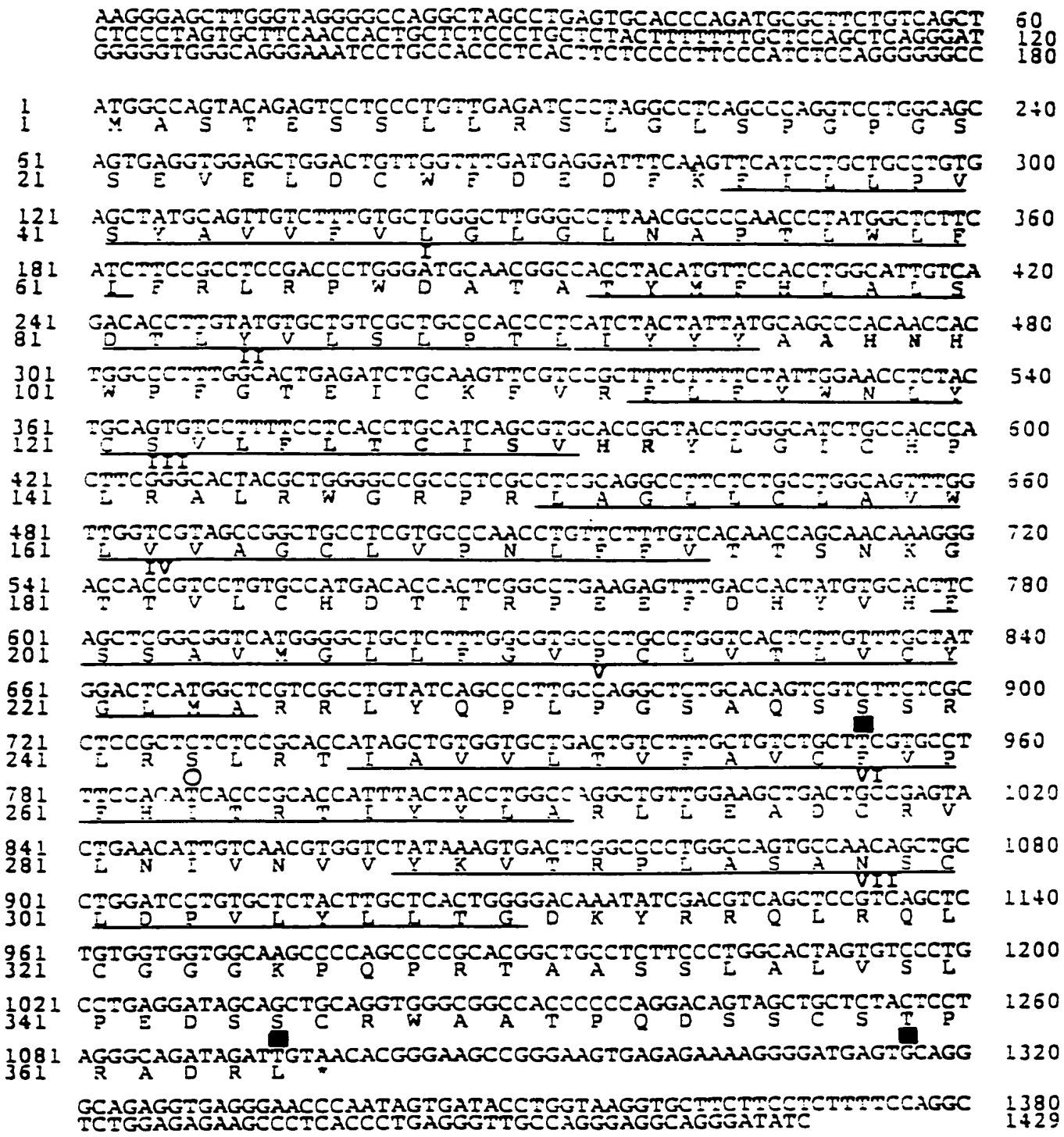
cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b) inserting the vector of step a in a suitable host cell;

c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;

d) recovering the receptor so obtained; and

e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.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CTCCCTAGTGCTTCAACCACTGCTCTCCCTGCTCTACTTTTTTTGCTCCAGCTCAGGGAT 120
GGGGGTGGGCAGGGAAATCCTGCCACCCTCACTTCTCCCCTTCCCATCTCCAGGGGGGCC 180
1 ATGGCCAGTACAGAGTCCTCCCTGTTGAGATCCCTAGGCCTCAGCCCAGGTCCTGGCAGC 240
1 M A S T E S S L L R S L G L S P G P G S
61 AGTGAGGTGGAGCTGGACTGTTGGTTTGATGAGGATTTCAAGTTCATCCTGCTGCCTGTG 300
21 S E V E L D C W F D E D F K F I L L P V
121 AGCTATGCAGTTGTCTTTGTGCTGGGCTTGGGCCTTAACGCCCCAACCCTATGGCTCTTC 360
41 S Y A V V F V L G L G L N A P T L W L F
181 ATCTTCCGCCTCCGACCCTGGGATGCAACGGCCACCTACATGTTCCACCTGGCATTGTCA 420
61 I F R L R P W D A T A T Y M F H L A L S
241 GACACCTTGTATGTGCTGTCGCTGCCCACCCTCATCTACTATTATGCAGCCCACAACCAC 480
81 D T L Y V L S L P T L I Y Y Y A A H N H
301 TGGCCCTTTGGCACTGAGATCTGCAAGTTCGTCCGCTTTCTTTTCTATTGGAACCTCTAC 540
101 W P F G T E I C K F V R F L F Y W N L Y
361 TGCAGTGTCCTTTTCCTCACCTGCATCAGCGTGCACCGCTACCTGGGCATCTGCCACCCA 600
121 C S V L F L T C I S V H R Y L G I C H P
421 CTTCGGGCACTACGCTGGGGCCGCCCTCGCCTCGCAGGCCTTCTCTGCCTGGCAGTTTGG 660
141 L R A L R W G R P R L A G L L C L A V W
481 TTGGTCGTAGCCGGCTGCCTCGTGCCCAACCTGTTCTTTGTCACAACCAGCAACAAAGGG 720
161 L V V A G C L V P N L F F V T T S N K G
541 ACCACCGTCCTGTGCCATGACACCACTCGGCCTGAAGAGTTTGACCACTATGTGCACTTC 780
181 T T V L C H D T T R P E E F D H Y V H F
601 AGCTCGGCGGTCATGGGGCTGCTCTTTGGCGTGCCCTGCCTGGTCACTCTTGTTTGCTAT 840
201 S S A V M G L L F G V P C L V T L V C Y
661 GGACTCATGGCTCGTCGCCTGTATCAGCCCTTGCCAGGCTCTGCACAGTCGTCTTCTCGC 900
221 G L M A R R L Y Q P L P G S A Q S S S R
721 CTCCGCTCTCTCCGCACCATAGCTGTGGTGCTGACTGTCTTTGCTGTCTGCTTCGTGCCT 960
241 L R S L R T I A V V L T V F A V C F V P
781 TTCCACATCACCCGCACCATTTACTACCTGGCCAGGCTGTTGGAAGCTGACTGCCGAGTA 1020
261 F H I T R T I Y Y L A R L L E A D C R V
841 CTGAACATTGTCAACGTGGTCTATAAAGTGACTCGGCCCCTGGCCAGTGCCAACAGCTGC 1080
281 L N I V N V V Y K V T R P L A S A N S C
901 CTGGATCCTGTGCTCTACTTGCTCACTGGGGACAAATATCGACGTCAGCTCCGTCAGCTC 1140
301 L D P V L Y L L T G D K Y R R Q L R Q L
961 TGTGGTGGTGGCAAGCCCCAGCCCCGCACGGCTGCCTCTTCCCTGGCACTAGTGTCCCTG 1200
321 C G G G K P Q P R T A A S S L A L V S L
1021 CCTGAGGATAGCAGCTGCAGGTGGGCGGCCACCCCCCAGGACAGTAGCTGCTCTACTCCT 1260
341 P E D S S C R W A A T P Q D S S C S T P
1081 AGGGCAGATAGATTGTAACACGGGAAGCCGGGAAGTGAGAGAAAAGGGGATGAGTGCAGG 1320
361 R A D R L *
GCAGAGGTGAGGGAACCCAATAGTGATACCTGGTAAGGTGCTTCTTCCTCTTTTCCAGGC 1380
TCTGGAGAGAAGCCCTCACCCTGAGGGTTGCCAGGGAGGCAGGGATATC 1429

FIG.1

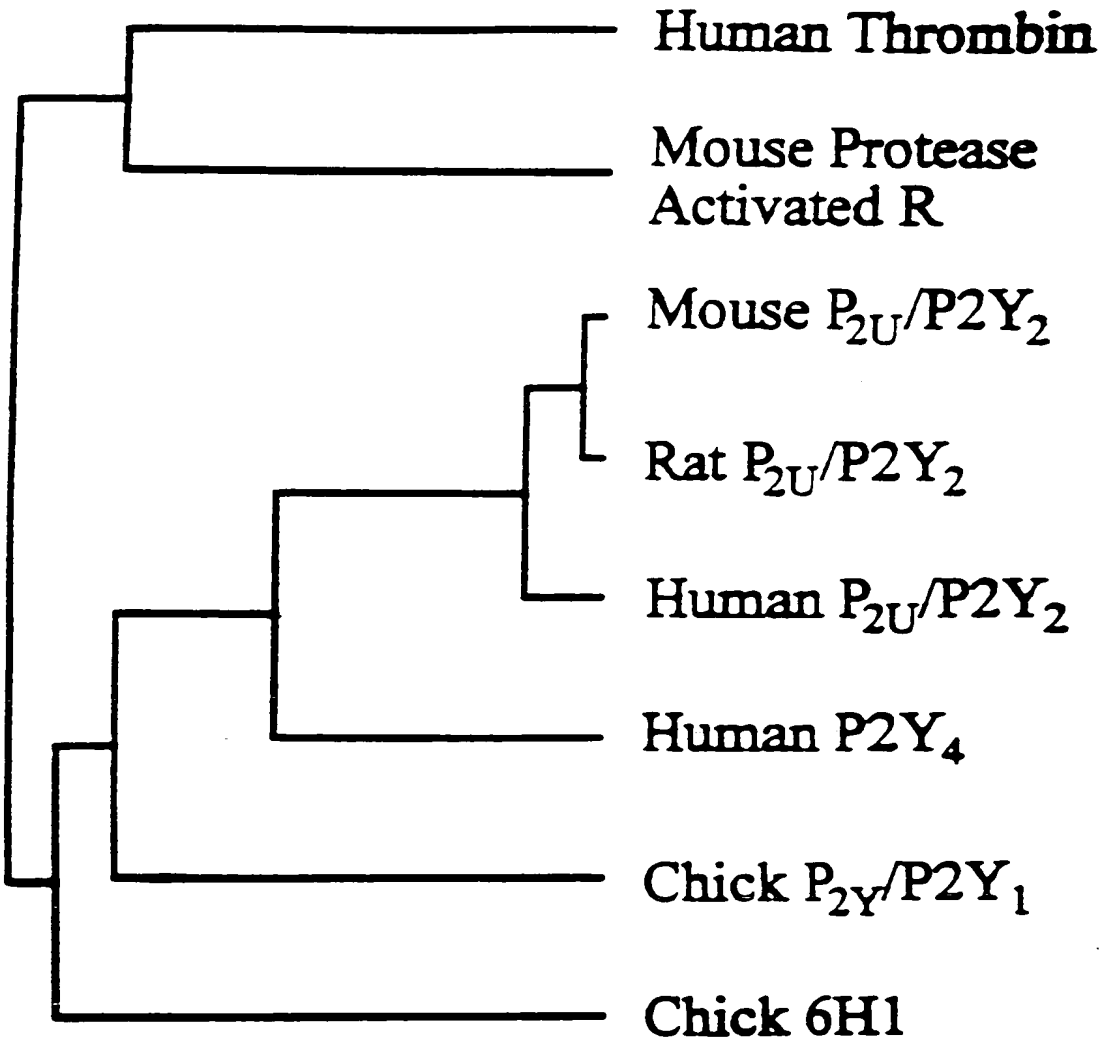

Human Thrombin
Mouse Protease Activated R
Mouse P_{2U}/P2Y_2
Rat P_{2U}/P2Y_2
Human P_{2U}/P2Y_2
Human P2Y_4
Chick P_{2Y}/P2Y_1
Chick 6H1

FIG.2

Expression of $P2Y_4$ receptors

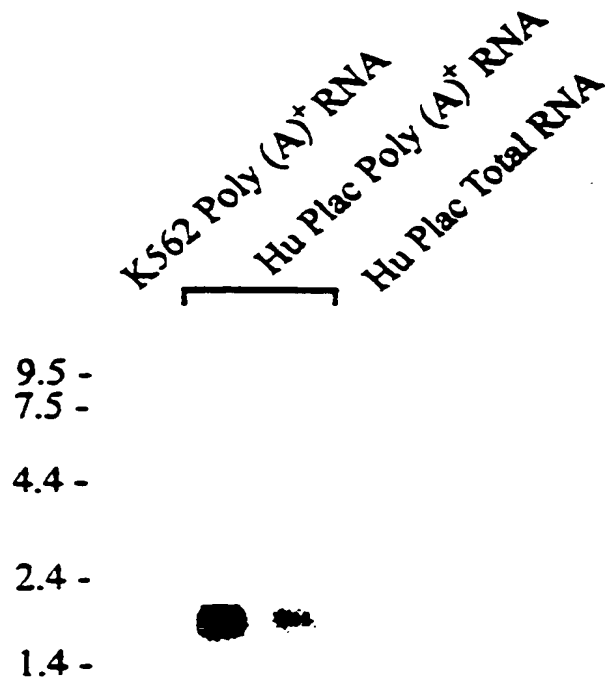


FIG.3

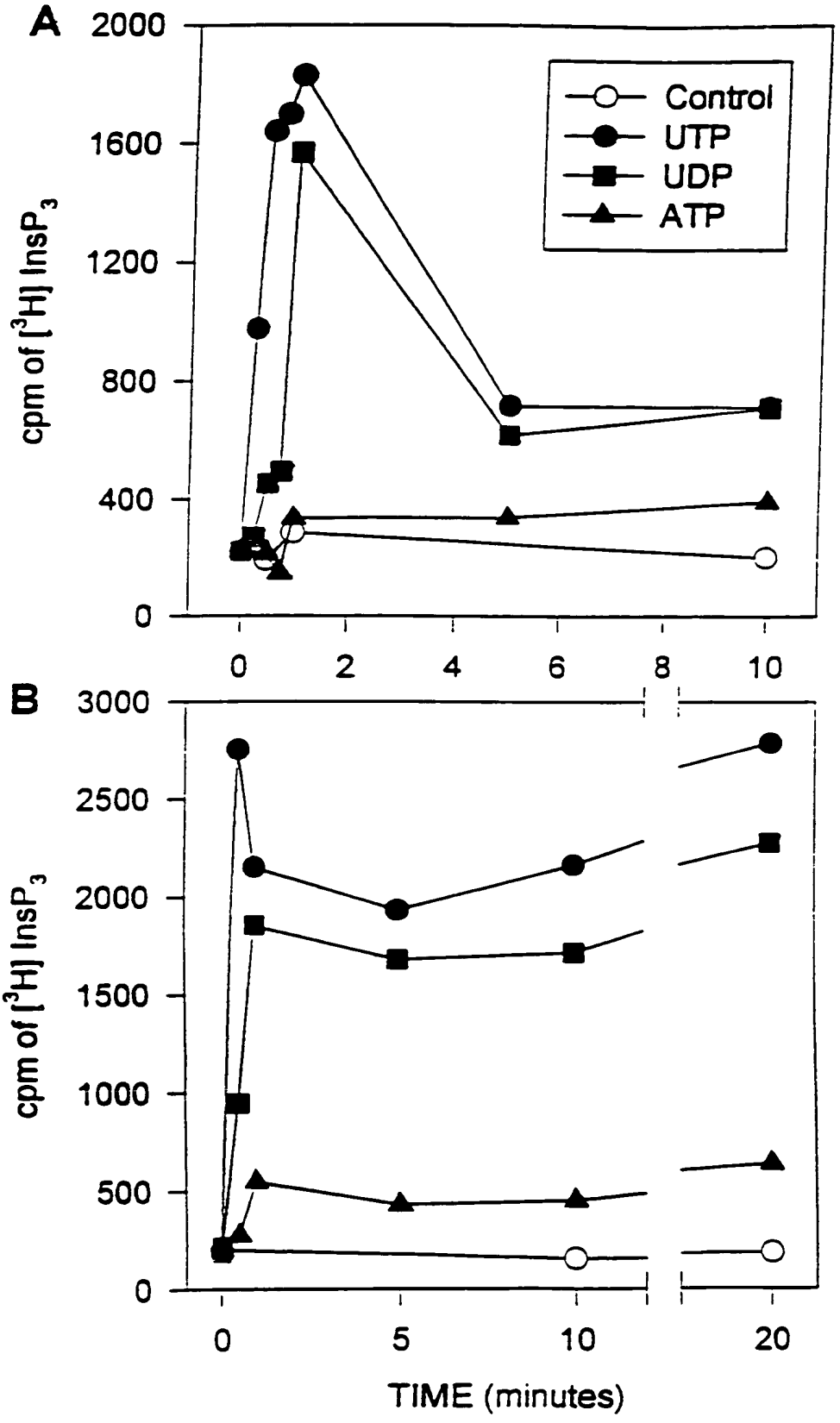

A
B
Control
UTP
UDP
ATP
cpm of [3H] InsP3
cpm of [3H] InsP3
TIME (minutes)
0
400
800
1200
1600
2000
500
1000
1500
2000
2500
3000
0
2
4
6
8
10
0
5
10
20

Fig. 4

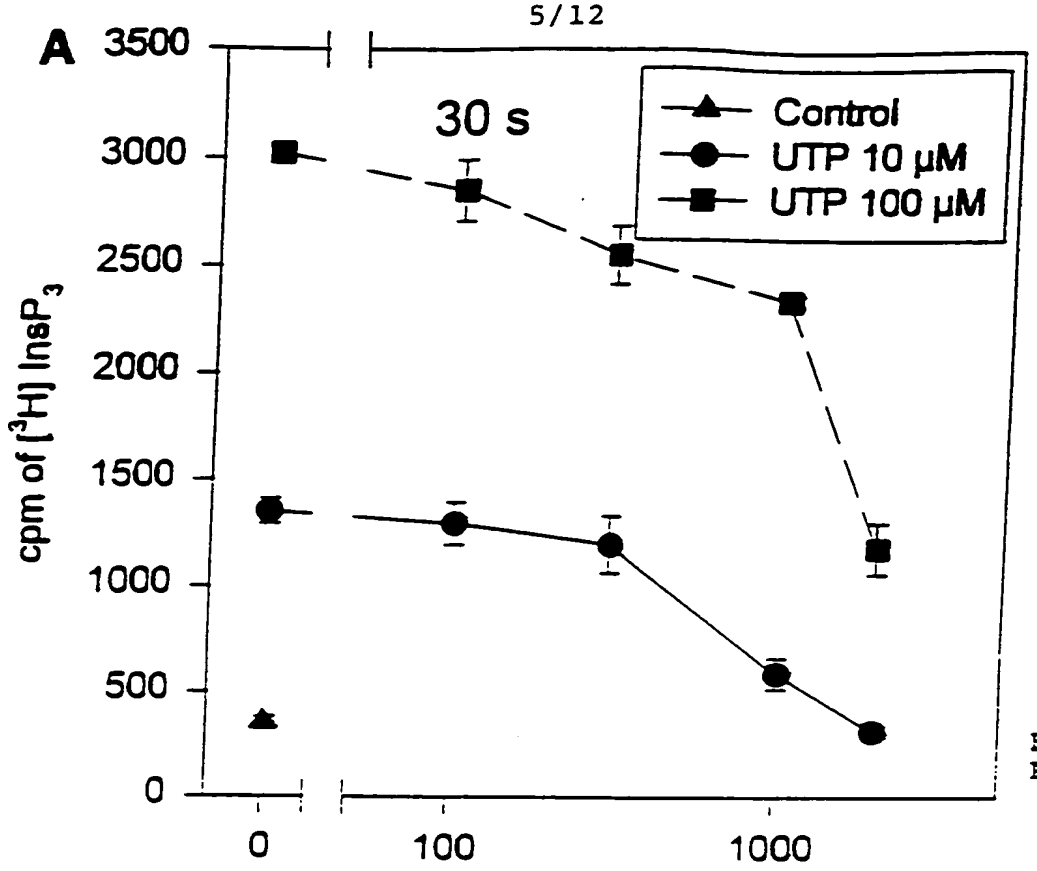


Fig. 5
Panel A

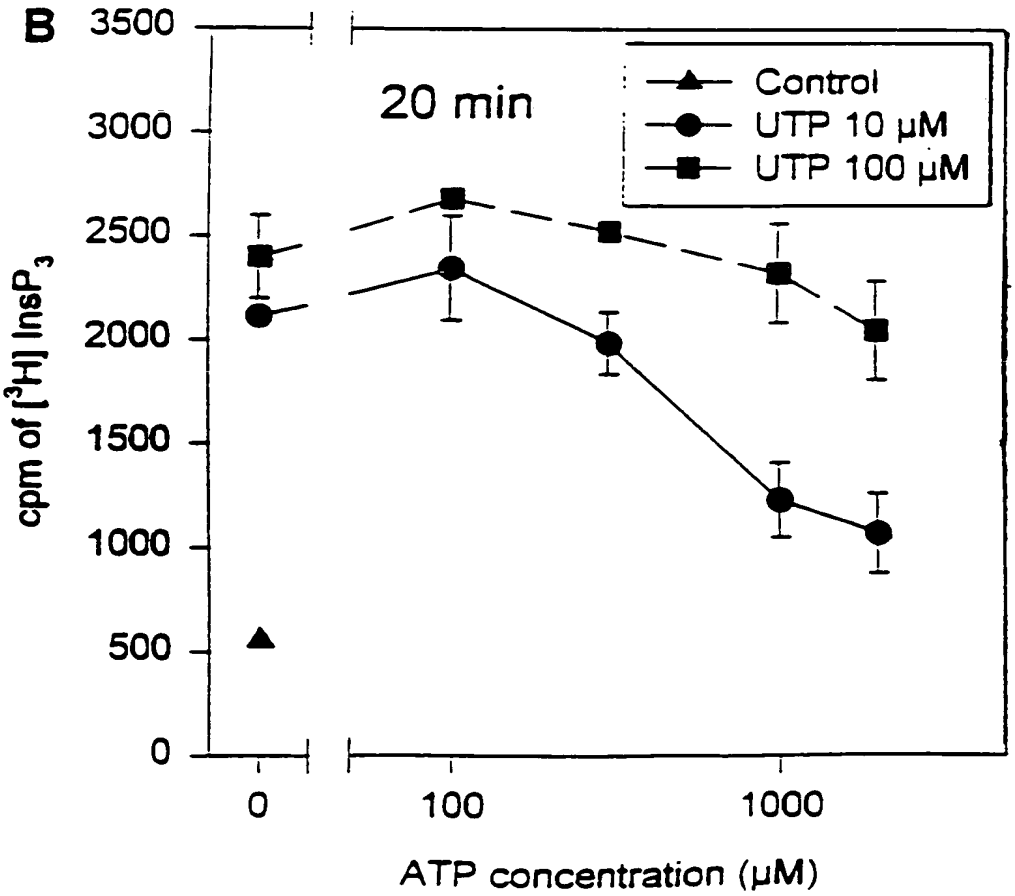


Fig. 5
Panel B

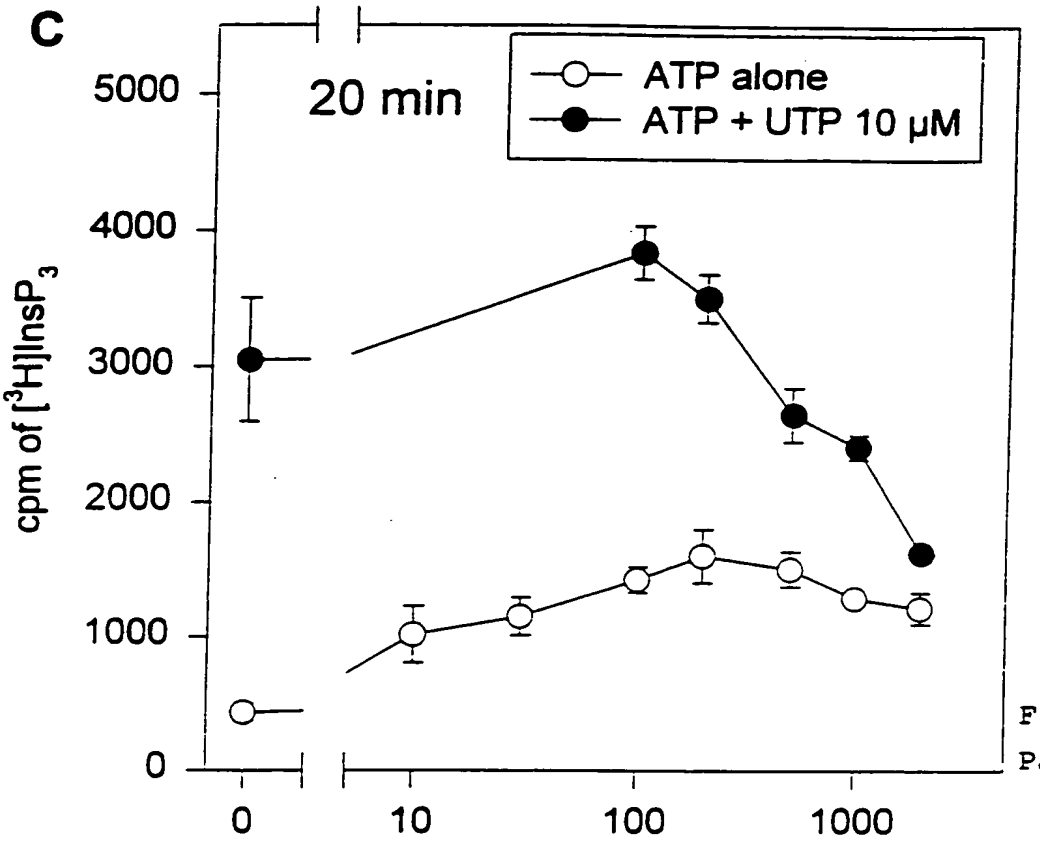
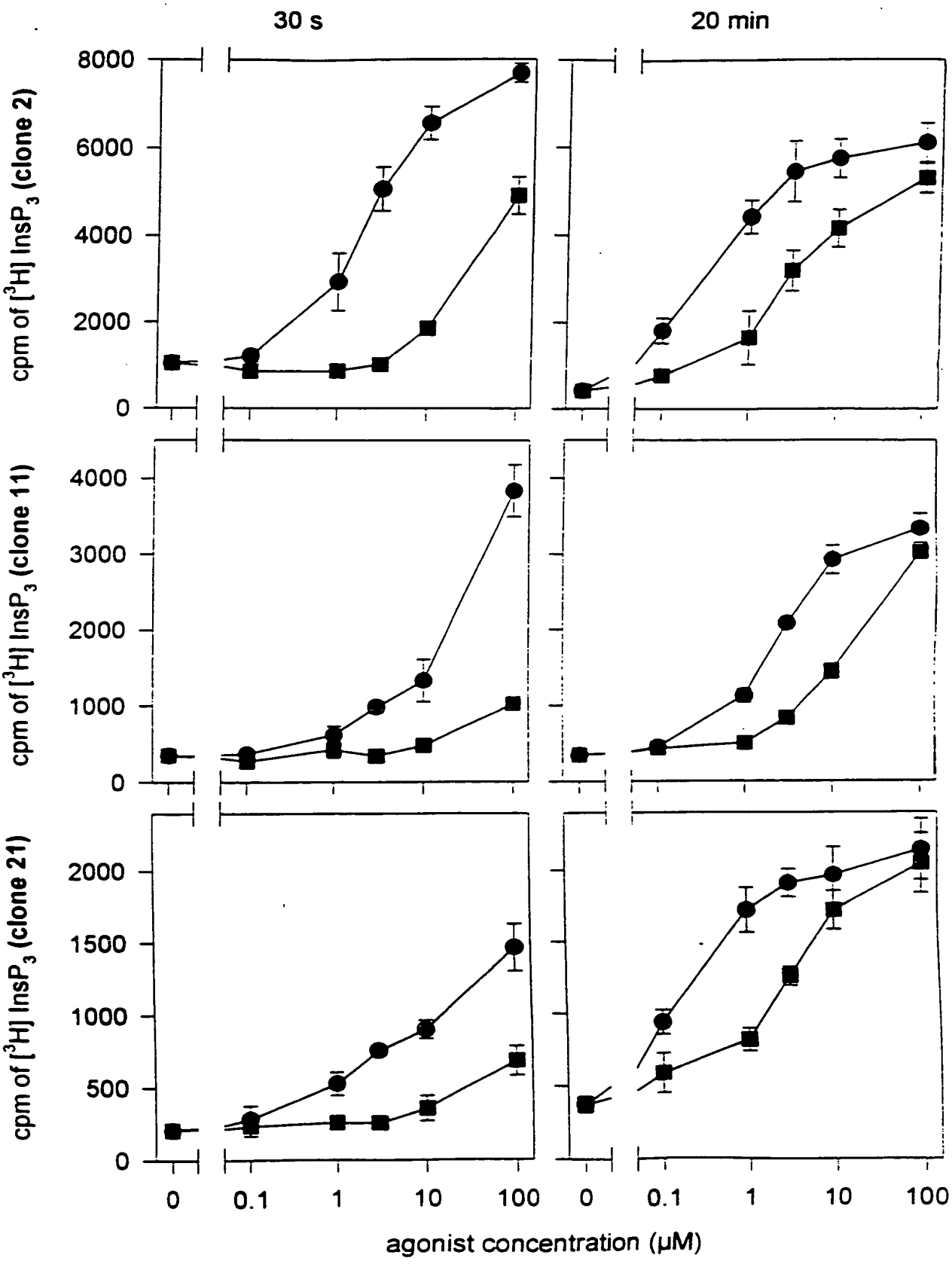

C
20 min
ATP alone
ATP + UTP 10 µM
cpm of [3H]InsP3
5000
4000
3000
2000
1000
0
0
10
100
1000

Fig. 5
Panel C


30 s
20 min
cpm of [3H] InsP3 (clone 2)
cpm of [3H] InsP3 (clone 11)
cpm of [3H] InsP3 (clone 21)
8000
6000
4000
2000
0
4000
3000
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0
2000
1500
1000
500
0
0
0.1
1
10
100
agonist concentration (μM)

Fig.6

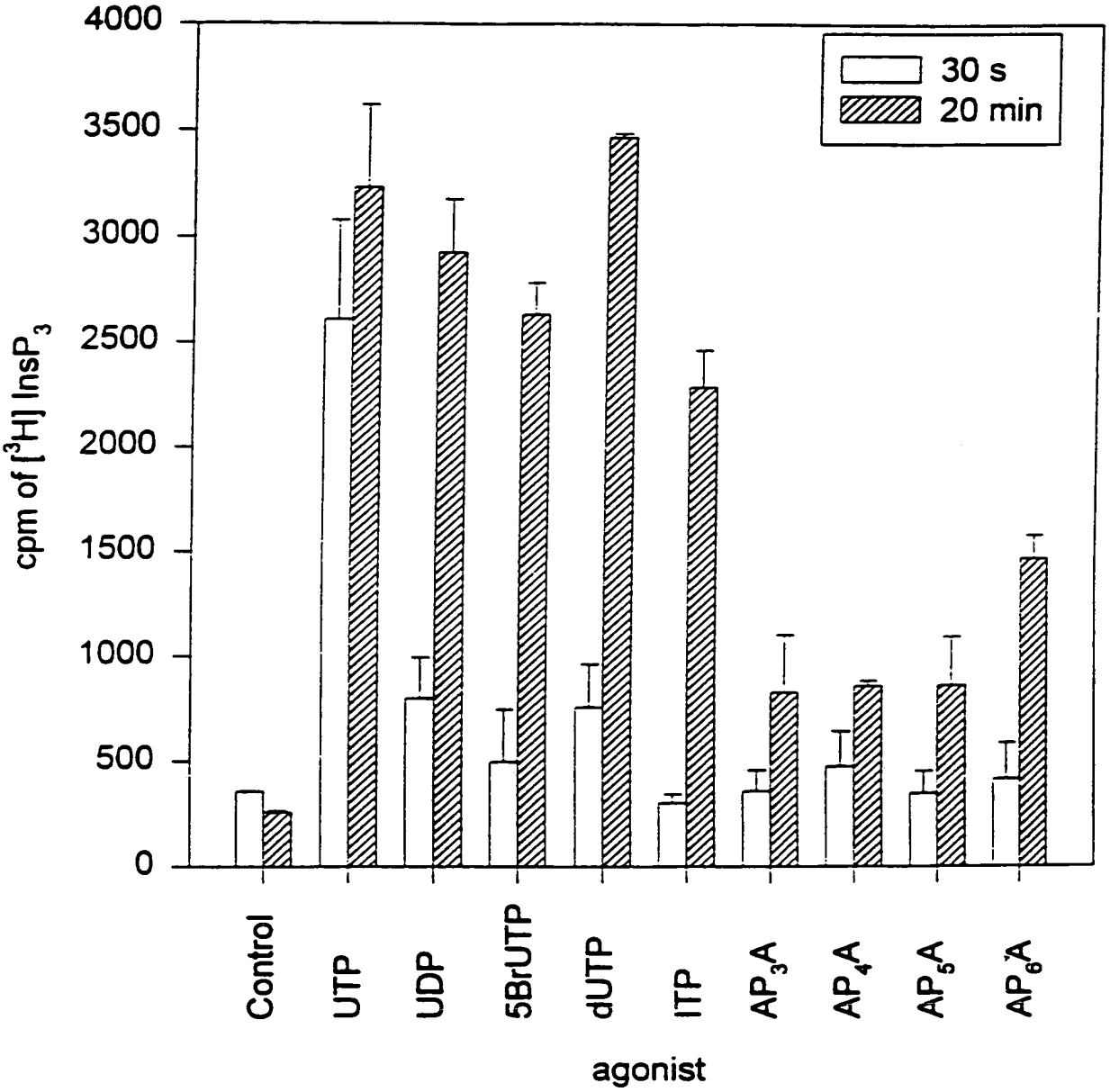
4000
3500
3000
2500
2000
1500
1000
500
0
cpm of [3H] InsP3
30 s
20 min
Control
UTP
UDP
5BrUTP
dUTP
ITP
AP3A
AP4A
AP5A
AP6A
agonist

Fig. 7

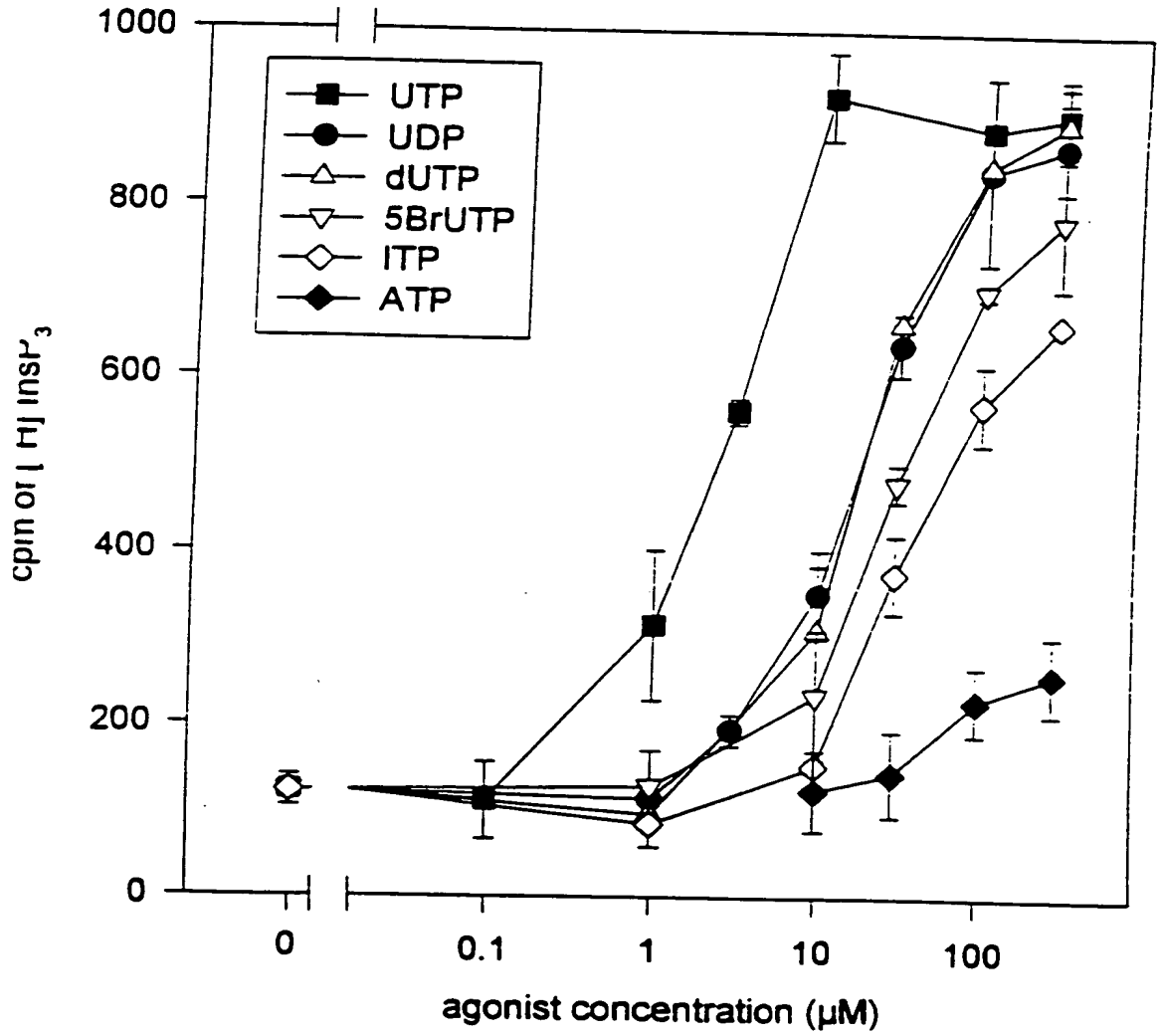
1000
800
600
400
200
0
cpm of [H] InsP3
UTP
UDP
dUTP
5BrUTP
ITP
ATP
0
0.1
1
10
100
agonist concentration (μM)

Fig.8

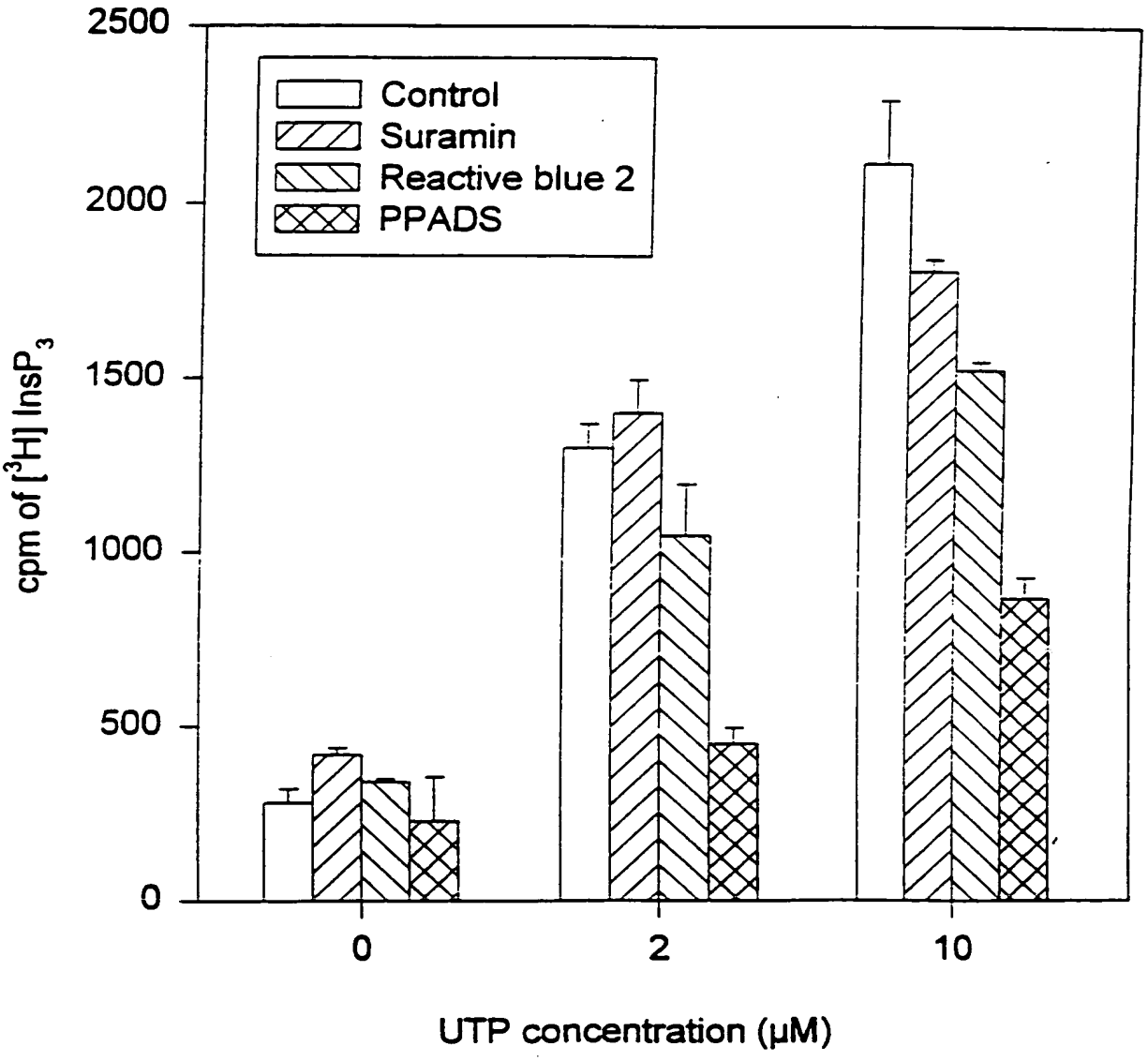
2500
2000
1500
1000
500
0
cpm of [3H] InsP3
Control
Suramin
Reactive blue 2
PPADS
0
2
10
UTP concentration (μM)

Fig. 9

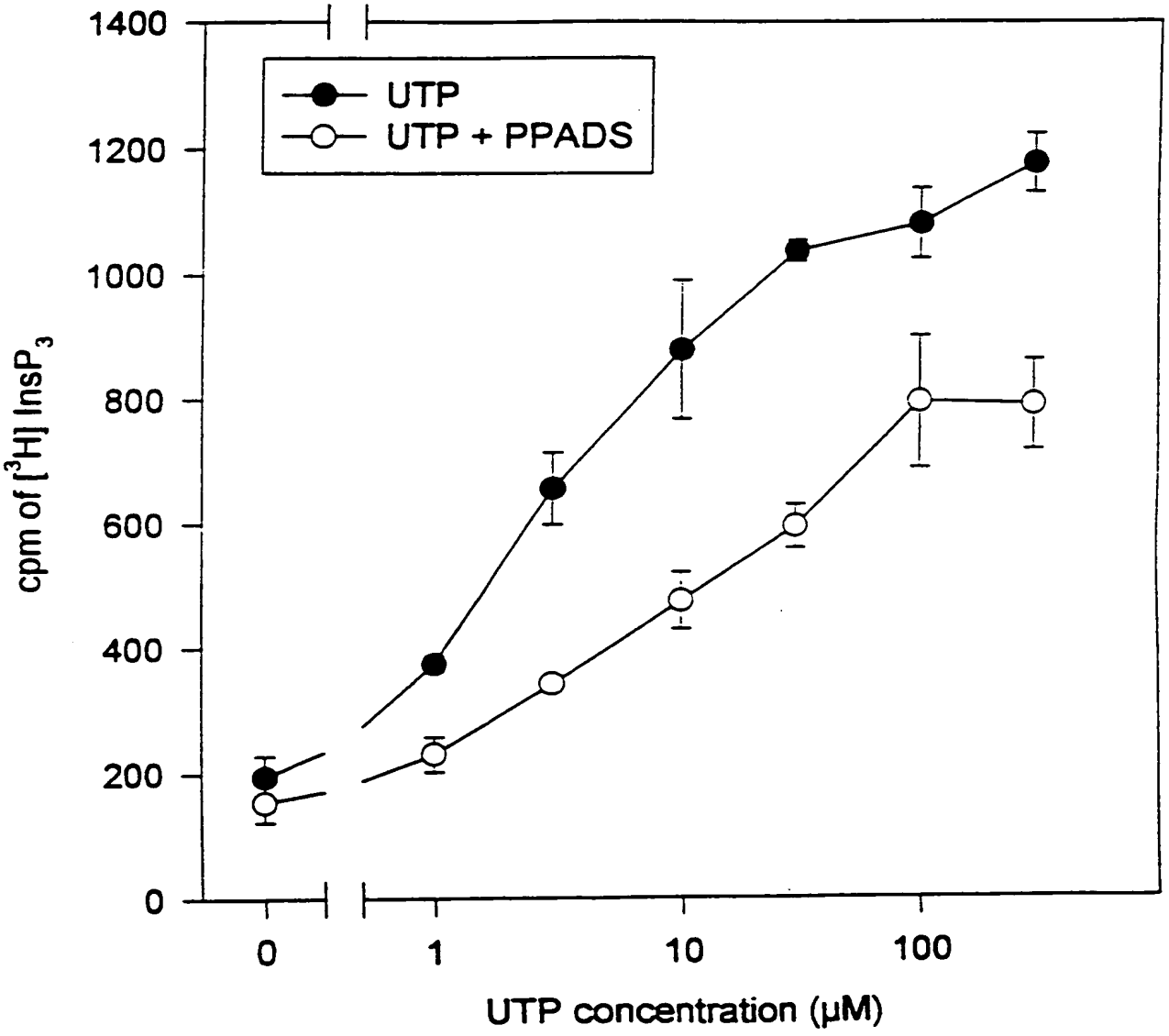
1400
1200
1000
800
600
400
200
0
UTP
UTP + PPADS
cpm of [3H] InsP3
0
1
10
100
UTP concentration (μM)

Fig. 10

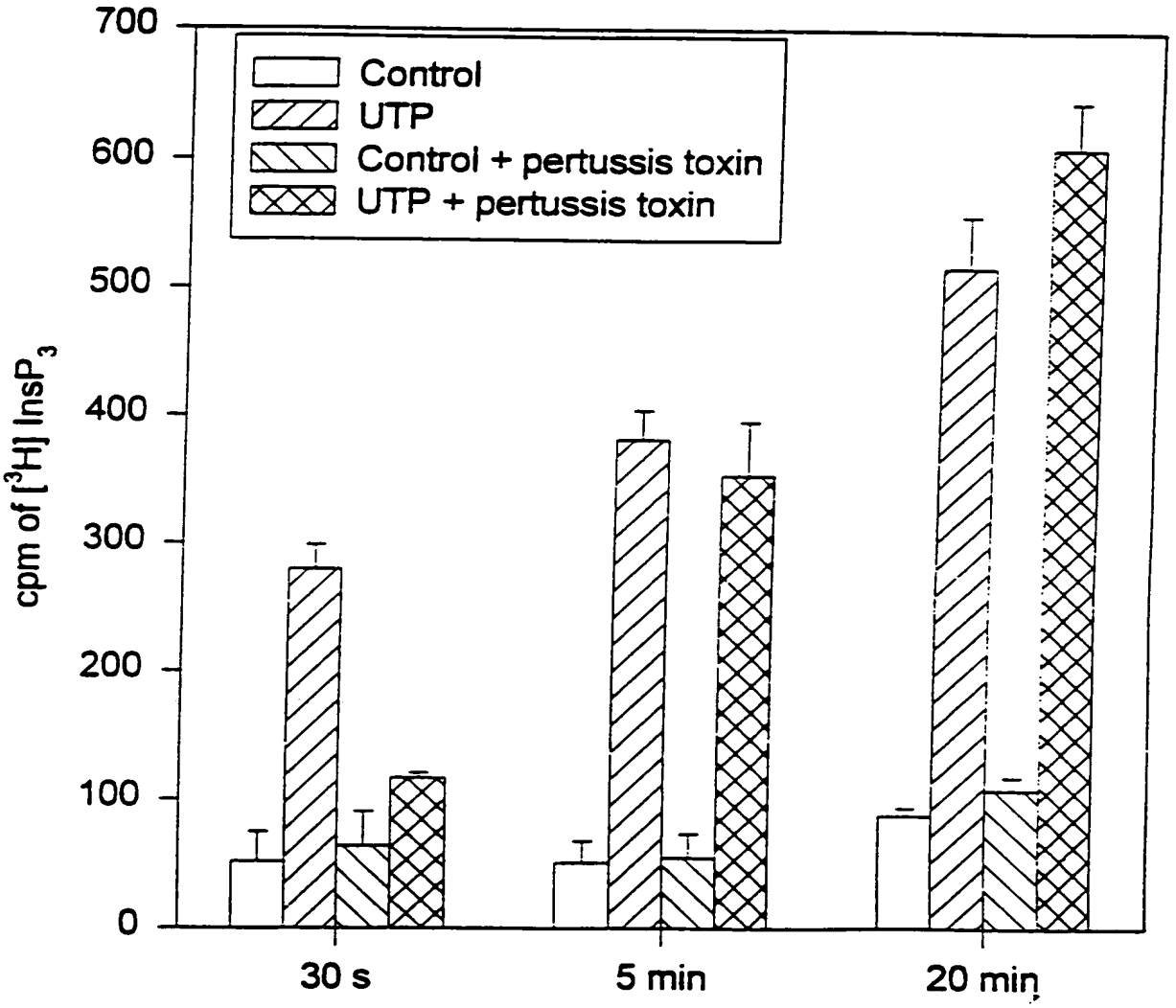
700
600
500
400
300
200
100
0
cpm of [^{3}H] InsP$_3$
Control
UTP
Control + pertussis toxin
UTP + pertussis toxin
30 s
5 min
20 min

Fig. 11

INTERNATIONAL SEARCH REPORT

Intern[illegible] Application No
PCT/BE 96/00123

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 C12N15/85 C12N15/86 C12N5/10
C12Q1/68 C07K16/28 A01K67/027 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q A01K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 91, April 1994, pages 3275-3279, XP000611412 PARR C E ET AL: "CLONING AND EXPRESSION OF A HUMAN P2U NUCLEOTIDE RECEPTOR, A TARGET FOR CYSTIC FIBROSIS PHARMACOTHERAPY" cited in the application see the whole document --- -/--	11-14, 16-19, 21-23

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 April 1997	02. 05. 97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 10538 A (UNIV NORTH CAROLINA ;UNIV MISSOURI (US); BOUCHER RICHARD C (US); W) 20 April 1995	14-19, 21-25
A	see the whole document	1-13, 26-28, 35,36, 38-40, 47-67, 76,77
A	TRENDS IN PHARMACOLOGICAL SCIENCES, vol. 16, April 1995, pages 133-139, XP002030122 BOARDER M. ET AL.: "G protein-coupled P2 purinoceptors: from molecular biology to functional responses" * see the whole document, esp. p. 137 *	1-77
A	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 16, 22 April 1994, pages 11830-11836, XP002030123 LAZAROWSKI E. AND HARDEN T.: "Identification of a uridine nucleotide-selective G-protein-linked receptor that activates phospholipase C" cited in the application see the whole document	1-77
P,X	FEBS LETTERS, vol. 384, no. 3, 22 April 1996, pages 260-264, XP002030124 STAM N. ET AL.: "Molecular cloning and characterization of a novel orphan receptor (P2p) expressed in human pancreas that shows high structural homology to the P2u purinoceptor" see the whole document	1-16,24, 25,27
P,X	THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 270, no. 52, 29 December 1995, pages 30849-30852, XP002030125 COMMUNI D. ET AL.: "Cloning and functional expression of a human uridine nucleotide receptor" see the whole document	1-23
T	WO 96 38558 A (INCYTE PHARMA INC) 5 December 1996 see the whole document	1-77

INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/BE 96/00123

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 70,73
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although these claims are directed to a method of treatment of (diagnostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern Application No
PCT/BE 96/00123

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9510538 A	20-04-95	AU 7965694 A	04-05-95
		US 5596088 A	21-01-97
		US 5607836 A	04-03-97
WO 9638558 A	05-12-96	AU 5972996 A	18-12-96
		AU 6032596 A	18-12-96
		WO 9638591 A	05-12-96

Rec'd on 21 May 1998

CLAIMS.

1. Receptor which has an amino acid sequence having more than 60% homology with the amino acid sequence shown in Figure 1.

2. Receptor according to claim 1, which has at least the amino acid sequence shown in Figure 1 or a portion thereof.

3. Receptor according to claim 1 or 2 having a preference for pyrimidine nucleotides over purine nucleotides.

4. Receptor according to claim 3, having at least a twofold preference, preferably tenfold to one hundredfold preference for pyrimidine nucleotides over purine nucleotides.

5. Receptor according to any of the claims 3 or 4, wherein the pyrimidine nucleotide is uridine triphosphate.

6. Receptor according to any of the claims 3 to 5, having a preference for UTP over UDP.

7. Receptor according to claim 5 being a high affinity UTP-specific receptor.

8. Receptor according to any of the preceding claims, belonging to the P2 receptor family.

9. Receptor according to any of the preceding claims, being a G protein-coupled receptor.

10. Receptor according to any of the preceding claims, being a human receptor.

11. Nucleic acid molecule encoding the receptor according to any of the preceding claims.

12. Nucleic acid molecule according to claim 11, wherein the nucleic acid molecule is DNA or RNA molecule.

13. DNA molecule according to claim 12, which is a cDNA molecule or a genomic DNA molecule.

14. Nucleic acid molecule according to any of the claims 11 to 13, having more than 60% homology to the DNA sequence shown in Figure 1.

15. DNA molecule according to claim 14, which has at least the DNA sequence as shown in figure 1 or a portion thereof.

16. Vector comprising the nucleic acid molecule according to any of the claims 11 to 15.

17. Vector according to claim 16, adapted for expression in a cell, which comprises the regulatory elements necessary for expression of the nucleic acid molecule in said cell operatively linked to the nucleic acid molecule according to any of the claims 11 to 15 as to permit expression thereof.

18. Vector of claim 17, wherein the cell is chosen among the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells.

19. Vector according to any of the claims 16 to 18, wherein the vector is a plasmid or a virus, preferably a baculovirus, an adenovirus or a Semliki Forest virus.

20. Vector according to claim 19, wherein the plasmid is pcDNA3-P2Y4.

21. Cell comprising the vector according to any of the claims 16 to 20.

22. Cell of claim 21, wherein the cell is a mammalian cell, preferably non neuronal in origin.

23. Cell of claim 21, wherein the cell is chosen among the group consisting of COS-7 cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 cells.

24. Nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the

nucleic acid molecule according to any of the claims 11 to 15.

25. Nucleic acid probe of claim 24, wherein the nucleic acid is DNA or RNA.

26. Antisense oligonucleotide having a sequence capable of specifically hybridizing to a mRNA molecule of claim 12, so as to prevent translation of the mRNA molecule.

27. Antisense oligonucleotide having a sequence capable of specifically hybridizing to the DNA molecule of claim 13.

28. Antisense oligonucleotide according to claim 26 or 27, comprising chemical analogs of nucleotides.

29. Ligand other than purine and pyridine nucleotides capable of binding to a receptor according to any of the claims 1 to 10.

30. Anti-ligand capable of competitively inhibiting the binding of the ligand according to claim 29 to the receptor according to any of the claims 1 to 10.

31. Ligand according to claim 29 which is an antibody.

32. Anti-ligand according to claim 30 which is an antibody.

33. Antibody according to claim 31 or 32, which is a monoclonal antibody.

34. Monoclonal antibody according to claim 33, directed to an epitope of the receptor according to any of the claims 1 to 10, present on the surface of a cell expressing said receptor.

35. Pharmaceutical composition comprising an amount of the oligonucleotide according to claim 26, effective to decrease activity of the receptor according to any of the claims 1 to 10 by passing through a cell membrane

14. Nucleic acid molecule according to any of the claims 11 to 13, having more than 60% homology to the DNA sequence shown in Figure 1.

15. DNA molecule according to claim 14, which has at least the DNA sequence as shown in figure 1 or a portion thereof.

16. Vector comprising the nucleic acid molecule according to any of the claims 11 to 15.

17. Vector according to claim 16, adapted for expression in a cell, which comprises the regulatory elements necessary for expression of the nucleic acid molecule in said cell operatively linked to the nucleic acid molecule according to any of the claims 11 to 15 as to permit expression thereof.

18. Vector of claim 17, wherein the cell is chosen among the group consisting of bacterial cells, yeast cells, insect cells or mammalian cells.

19. Vector according to any of the claims 16 to 18, wherein the vector is a plasmid or a virus, preferably a baculovirus, an adenovirus or a Semliki Forest virus.

20. Vector according to claim 19, wherein the plasmid is pcDNA3-P2Y4.

21. Cell comprising the vector according to any of the claims 16 to 20.

22. Cell of claim 21, wherein the cell is a mammalian cell, preferably non neuronal in origin.

23. Cell of claim 21, wherein the cell is chosen among the group consisting of COS-7 cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 cells.

24. Nucleic acid probe comprising a nucleic acid molecule of at least 15 nucleotides capable of specifically hybridizing with a unique sequence included within the

nucleic acid molecule according to any of the claims 11 to 15.

25. Nucleic acid probe of claim 24, wherein the nucleic acid is DNA or RNA.

26. Antisense oligonucleotide having a sequence capable of specifically hybridizing to a mRNA molecule of claim 12, so as to prevent translation of the mRNA molecule.

27. Antisense oligonucleotide having a sequence capable of specifically hybridizing to the DNA molecule of claim 13.

28. Antisense oligonucleotide according to claim 26 or 27, comprising chemical analogs of nucleotides.

29. Ligand other than purine and pyridine nucleotides capable of binding to a receptor according to any of the claims 1 to 10.

30. Anti-ligand capable of competitively inhibiting the binding of the ligand according to claim 29 to the receptor according to any of the claims 1 to 10.

31. Ligand according to claim 29 which is an antibody.

32. Anti-ligand according to claim 30 which is an antibody.

33. Antibody according to claim 31 or 32, which is a monoclonal antibody.

34. Monoclonal antibody according to claim 33, directed to an epitope of the receptor according to any of the claims 1 to 10, present on the surface of a cell expressing said receptor.

35. Pharmaceutical composition comprising an amount of the oligonucleotide according to claim 26, effective to decrease activity of the receptor according to any of the claims 1 to 10 by passing through a cell membrane

and binding specifically with mRNA encoding said receptor in the cell so as to prevent its translation, and a pharmaceutically acceptable carrier capable of passing through a cell membrane.

36. Pharmaceutical composition of claim 35, wherein the oligonucleotide is coupled to a substance which inactivates mRNA.

37. Pharmaceutical composition of claim 36, wherein the substance which inactivates mRNA is a ribozyme.

38. Pharmaceutical composition according to any of the claims 35 to 37, wherein the pharmaceutically acceptable carrier comprises a structure which binds to a receptor on a cell capable of being taken up by cell after binding to the structure.

39. Pharmaceutical composition of claim 38, wherein the structure of the pharmaceutically acceptable carrier is capable of binding to a receptor which is specific for a selected cell type.

40. Pharmaceutical composition which comprises an effective amount of the anti-ligand of claim 30, effective to block binding of a ligand to the receptor according to any of the claims 1 to 10 and a pharmaceutically acceptable carrier.

41. Transgenic non human mammal expressing the nucleic acid molecule according to any of the claims 11 to 15.

42. Transgenic non human mammal comprising a homologous recombination knockout of the native receptor according to any of the claims 1 to 10.

43. Transgenic non human mammal whose genome comprises antisense nucleic acid complementary to the nucleic acid molecule according to any of the claims 11 to 15 so

placed as to be transcripted into antisense mRNA which is complementary to the mRNA of claim 12 and which hybridizes to said mRNA thereby reducing its translation.

44. Transgenic non human mammal according to any of the claims 41 to 43, wherein the nucleic acid according to any of the claims 11 to 15 additionally comprises an inducible promoter.

45. Transgenic non human mammal according to any of the claims 41 to 44, wherein the nucleic acid according to claim 11 to 15 additionally comprises tissue specific regulatory elements.

46. Transgenic non human mammal according to any of the claims 41 to 45, which is a mouse.

47. Method for determining whether a ligand can specifically bind to a receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting binding of ligand to such receptor and detecting the presence of any such ligand bound specifically to said receptor, thereby determining whether the ligand binds specifically to said receptor.

48. Method for determining whether a ligand can specifically bind to the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the ligand with the membrane fraction under conditions permitting binding of the ligand to such receptor and detecting the presence of any ligand bound to said receptor, thereby determining whether the compound is capable of specifically binding to said

receptor.

49. Method for determining whether a ligand is an agonist of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand under conditions permitting the activation of a functional receptor response from the cell and detecting by means of a bio-assay, such as a modification in a second messenger concentration or a modification in the cellular metabolism, an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

50. Method for determining whether a ligand is an agonist of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in the production of a second messenger, an increase in the receptor activity, thereby determining whether the ligand is a receptor agonist.

51. Method for determining whether a ligand is an antagonist of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in a second messenger concentration or a modification in the cellular metabolism,

a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

52. Method for determining whether a ligand is an antagonist of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with the ligand in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response and detecting by means of a bio-assay, such as a modification in a second messenger concentration, a decrease in the receptor activity, thereby determining whether the ligand is a receptor antagonist.

53. A method according to any of the claims 47 to 50, wherein the second messenger assay comprises measurement of intra-cellular cAMP, intra-cellular Inositol phosphate, intra-cellular diacylglycerol concentration or intra-cellular calcium mobilization.

54. Method according to any of the preceding claims 47 to 53, wherein the cell is a mammalian cell, preferably non neuronal in origin, and chosen among the group consisting of COS-7 cells, CHO cells, LM(tk-) cells, NIH-3T3 cells or 1321N1 cells.

55. Method according to any of the preceding claims 47 to 54, wherein the ligand is not previously known.

56. Ligand detected by the method according to any of the preceding claims 47 to 55.

57. Pharmaceutical composition which comprises the ligand according to claim 56 and a pharmaceutically acceptable carrier.

58. Method of screening drugs to identify drugs

which specifically bind to the receptor according to any of the claims 1 to 10 on the surface of the cell, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs under conditions permitting binding of said drugs to the receptor, and determining those drugs which specifically bind to the transfected cell, thereby identifying drugs which specifically bind to the receptor.

59. Method of screening drugs to identify drugs which specifically bind to the receptor according to any of the claims 1 to 10 on the surface of the cell, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cells extract, contacting the membrane fraction with a plurality of drugs and determining those drugs which bind to the transfected cell, thereby identifying drugs which specifically bind to said receptor.

60. Method of screening drugs to identify drugs which act as agonists of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs under conditions permitting the activation of a functional receptor response, and determining those drugs which activate such receptor using a bio-assay, such as a modification in a second messenger concentration or modification in the cellular metabolism, thereby identifying drugs which act as receptor agonists.

61. Method of screening drugs to identify drugs which act as agonists of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from

cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs under conditions permitting the activation of a functional receptor response, and determining those drugs which activate such receptor using a bio-assay, such as a modification in a second messenger concentration, thereby identifying drugs which act as receptor agonists.

62. Method of screening drugs to identify drugs which act as antagonists of the receptor according to any of the claims 1 to 10, which comprises contacting a cell transfected with a vector expressing the nucleic acid molecule encoding said receptor with a plurality of drugs in the presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response, and determining those drugs which inhibit the activation of the receptor using a bio-assay, such as a modification in a second messenger concentration or modification in the cellular metabolism, thereby identifying drugs which act as receptor antagonists.

63. Method of screening drugs to identify drugs which act as antagonists of the receptor according to any of the claims 1 to 10, which comprises preparing a cell extract from cells transfected with a vector expressing the nucleic acid molecule encoding said receptor, isolating a membrane fraction from the cell extract, contacting the membrane fraction with a plurality of drugs in presence of a known receptor agonist, under conditions permitting the activation of a functional receptor response, and determining those drugs which inhibit the activation of the receptor using a bio-assay, such as a modification in a second messenger

concentration, thereby identifying drugs which act as receptor antagonists.

64. Drug detected by any of the methods according to claims 58 to 63.

65. Pharmaceutical composition comprising a drug according to claim 64.

66. Method of detecting the expression of the receptor according to any of the claims 1 to 10, by detecting the presence of mRNA coding said receptor, which comprises obtaining total RNA or total mRNA from the cell and contacting the RNA or mRNA so obtained with the nucleic acid probe according to claim 24 under hybridizing conditions, and detecting the presence of mRNA hybridized to the probe, thereby detecting the expression of the receptor by the cell.

67. Method of detecting the presence of the receptor according to any of the claims 1 to 10 on the surface of a cell, which comprises contacting the cell with the antibody of claim 31 under conditions permitting binding of the antibody to the receptor, and detecting the presence of the antibody bound to the cell, thereby detecting the presence of the receptor on the surface of the cell.

68. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 10, which comprises producing a transgenic non human mammal according to any of the claims 41 to 46 whose levels of receptor expression are varied by use of an inducible promoter which regulates the receptor expression.

69. Method of determining the physiological effects of expressing varying levels of the receptor according to any of the claims 1 to 10, which comprises producing a panel of transgenic non human mammals according

to any of the claims 41 to 46, each expressing a different amount of said receptor.

70. Method for identifying an antagonist of the receptor according to any of the claims 1 to 10 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by decreasing the activity of the receptor, which comprises administering the antagonist to a transgenic non human mammal according to any of the claims 40 to 45 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the transgenic non human mammal as a result of receptor activity, thereby identifying the antagonist.

71. Antagonist identified by the method of claim 70.

72. Pharmaceutical composition comprising an antagonist according to claim 71 and a pharmaceutically acceptable carrier.

73. Method for identifying an agonist of the receptor according to any of the claims 1 to 10 capable of alleviating an abnormality in a subject wherein the abnormality is alleviated by activation of said receptor, which comprises administering the agonist to a transgenic non human mammal according to any of the claims 41 to 46 and determining whether the antagonist alleviates the physical and behavioural abnormalities displayed by the transgenic non human mammal, the alleviation of the abnormalities indicating the identification of the agonist.

74. Agonist identified by the method of claim 73.

75. Pharmaceutical composition comprising an agonist according to claim 74 and a pharmaceutically acceptable carrier.

76. Method for diagnosing a predisposition to a

disorder associated with the activity of a specific allele of the receptor according to any of the claims 1 to 10, which comprises:

a) obtaining nucleic acid molecules of subjects suffering from said disorder;

b) performing a restriction digest of said nucleic acid molecules with a panel of restriction enzymes;

c) electrophoretically separating the resulting nucleic acid fragments on a sized gel;

d) contacting the resulting gel with a nucleic acid probe capable of specifically hybridizing to said nucleic acid molecule and labelled with a detectable marker;

e) detecting labelled bands which have hybridized to the said nucleic acid molecule labelled with a detectable marker to create a unique band pattern specific to subjects suffering from said disorder;

f) preparing nucleic acid molecules obtained for diagnosis by step a-e; and

g) comparing the unique band pattern specific to the nucleic acid molecule of subjects suffering from the disorder from step e and the nucleic acid molecule obtained for diagnosis from step f to determine whether the patterns are the same or different and to diagnose thereby predisposition to the disorder if the patterns are the same.

77. Method of preparing the purified receptor according to any of the claims 1 to 10, which comprises:

a) constructing a vector adapted for expression in a cell which comprises the regulatory elements necessary for the expression of nucleic acid molecules in the cell operatively linked to nucleic acid molecule encoding said receptor so as to permit expression thereof, wherein the

cell is selected from the group consisting of bacterial cells, yeast cells, insect cells and mammalian cells;

b) inserting the vector of step a in a suitable host cell;

c) incubating the cell of step b under conditions allowing the expression of the receptor according to the invention;

d) recovering the receptor so obtained; and

e) purifying the receptor so recovered, thereby preparing an isolated receptor according to the invention.